PATENT APPLICATION

MAMMALIAN CYTOKINES; RECEPTORS;
RELATED REAGENTS AND METHODS

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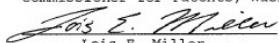
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MAMMALIAN CYTOKINES; RECEPTORS;
RELATED REAGENTS AND METHODS

This filing is a U.S. Utility Patent Application, which
5   claims the benefit of filing of U.S. Provisional Patent
Application, U.S.S.N. 60/179,638, filed February 2, 2000.

FIELD OF THE INVENTION

The present invention relates to compositions and methods
10   for affecting mammalian physiology, including morphogenesis or
immune system function. In particular, it provides nucleic
acids, proteins, and antibodies which regulate development
and/or the immune system; and provides functional details on
ligand-receptor pairing. Diagnostic and therapeutic uses of
15   these materials are also disclosed.

BACKGROUND OF THE INVENTION

Recombinant DNA technology refers generally to techniques
of integrating genetic information from a donor source into
20   vectors for subsequent processing, such as through
introduction into a host, whereby the transferred genetic
information is copied and/or expressed in the new environment.
Commonly, the genetic information exists in the form of
complementary DNA (cDNA) derived from messenger RNA (mRNA)
25   coding for a desired protein product. The carrier is
frequently a plasmid having the capacity to incorporate cDNA
for later replication in a host and, in some cases, actually
to control expression of the cDNA and thereby direct synthesis
of the encoded product in the host.

30   For some time, it has been known that the mammalian
immune response is based on a series of complex cellular
interactions, called the "immune network". Recent research
has provided new insights into the inner workings of this
network. While it remains clear that much of the immune
35   response does, in fact, revolve around the network-like

interactions of lymphocytes, macrophages, granulocytes, and other cells, immunologists now generally hold the opinion that soluble proteins, known as lymphokines, cytokines, or monokines, play critical roles in controlling these cellular

5   interactions. Thus, there is considerable interest in the isolation, characterization, and mechanisms of action of cell modulatory factors, an understanding of which will lead to significant advancements in the diagnosis and therapy of numerous medical abnormalities, e.g., immune system disorders.

10   Lymphokines apparently mediate cellular activities in a variety of ways. They have been shown to support the proliferation, growth, and/or differentiation of pluripotent hematopoietic stem cells into vast numbers of progenitors comprising diverse cellular lineages which make up a complex

15   immune system. Proper and balanced interactions between the cellular components are necessary for a healthy immune response. The different cellular lineages often respond in a different manner when lymphokines are administered in conjunction with other agents.

20   Cell lineages especially important to the immune response include two classes of lymphocytes: B-cells, which can produce and secrete immunoglobulins (proteins with the capability of recognizing and binding to foreign matter to effect its removal), and T-cells of various subsets that

25   secrete lymphokines and induce or suppress the B-cells and various other cells (including other T-cells) making up the immune network. These lymphocytes interact with many other cell types.

       Another important cell lineage is the mast cell (which

30   has not been positively identified in all mammalian species), which is a granule-containing connective tissue cell located proximal to capillaries throughout the body. These cells are found in especially high concentrations in the lungs, skin, and gastrointestinal and genitourinary tracts. Mast cells

play a central role in allergy-related disorders, particularly anaphylaxis as follows: when selected antigens crosslink one class of immunoglobulins bound to receptors on the mast cell surface, the mast cell degranulates and releases mediators,

5   e.g., histamine, serotonin, heparin, and prostaglandins, which cause allergic reactions, e.g., anaphylaxis.

Research to better understand and treat various immune disorders has been hampered by the general inability to maintain cells of the immune system in vitro. Immunologists

10 have discovered that culturing many of these cells can be accomplished through the use of T-cell and other cell supernatants, which contain various growth factors, including many of the lymphokines.

The interleukin-1 family of proteins includes the IL-1α,

15 the IL-1β, the IL-1RA (IL-1 receptor antagonist), and recently the IL-1γ (also designated Interferon-Gamma Inducing Factor, IGIF). This related family of genes have been implicated in a broad range of biological functions, e.g., inflammatory, infectious, or other immunological responses. See Dinarello

20 (1994) FASEB J. 8:1314-1325; Dinarello (1991) Blood 77:1627-1652; and Okamura, et al. (1995) Nature 378:88-91.

In addition, various growth and regulatory factors exist which modulate morphogenetic development. This includes, e.g., the Toll ligands, which signal through binding to

25 receptors which share structural, and mechanistic, features characteristic of the IL-1 receptors. See, e.g., Lemaitre, et al. (1996) Cell 86:973-983; and Belvin and Anderson (1996) Ann. Rev. Cell & Develop. Biol. 12:393-416.

From the foregoing, it is evident that the discovery and

30 development of new soluble proteins, including ones similar to lymphokines, should contribute to new therapies for a wide range of degenerative or abnormal conditions which directly or indirectly involve development, differentiation, or function, e.g., of the immune system and/or hematopoietic cells. In

particular, the discovery and understanding of novel
lymphokine-like molecules which enhance or potentiate the
beneficial activities of other lymphokines would be highly
advantageous. The present invention provides new interleukin-
1 like compositions and related compounds, and methods for
their use.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows an alignment between various IL-1 family
members. Positions refer to the alignment and are not residue
numbers. IL-1α is SEQ ID NO:5, IL-1β is SEQ ID NO:6, IL-1RA
is SEQ ID NO:7, mIL-1γ is SEQ ID NO:8, mIL-1ε is SEQ ID NO:9,
and mIL-1δ is SEQ ID NO:10.

SUMMARY OF THE INVENTION

The present invention is based on the discovery,
purification, and characterization of the biological
activities of novel interleukin-1 like molecules, designated
interleukin-1δ (IL-1δ) and interleukin-1ε (IL-1ε). IL-1δ and
IL-1ε exhibit both structural and sequence similarity, e.g.,
by homology comparison, to known members of the IL-1 family of
molecules. In addition, part of the receptor for these
molecules has been defined, the IL-1R related protein 2 (also
designated or IL-1R6), and the two molecules are agonist (IL-
1ε) and antagonist (IL-1δ). Thus, the receptor/ligand matching
provides a similar family to the IL-1α/IL-1β/IL-1RA signaling
system.

The present invention provides various methods of
producing a ligand:receptor complex, comprising contacting: a
substantially pure or recombinant mammalian IL-1δ or IL-1ε with
a receptor comprising the IL-1R6 receptor subunit; or a
mammalian IL-1δ or IL-1ε with a receptor comprising a
substantially pure or recombinant IL-1R6 receptor subunit;
thereby allowing the complex to form. Such methods include

those in which: the complex results in modulation of NFκB activation; the receptor is on a cell; the complex formation results in a physiological change in the cell expressing the receptor; the contacting is in combination with an anti-

5   inflammatory agent; or the contacting allows quantitative detection of the ligand. In other methods, the receptor is on a skin cell.

Other methods include methods of modulating physiology or development of an IL-1R6 receptor expressing cell comprising

10 contacting the cell to an exogenous agonist or antagonist of a mammalian IL-1δ or IL-1ε. In some embodiments: the antagonist is: an antibody which: neutralizes the mammalian IL-1δ; or neutralizes the mammalian IL-1ε; or a mutein of the IL-1δ or IL-1ε; or the physiology is selected from: proliferation;

15 tissue remodeling; or production of inflammatory mediators, including cytokines, chemokines, or adhesion molecules; or the modulating is specific for epithelial cells and not endothelial cells. In other embodiments, the antagonist is an antibody and the physiology is an inflammatory response; the

20 modulating is specific for Th2 cells and not Th1 cells; or the modulating is blocking, and the physiology is an inflammatory response.

Other aspects of the invention provide methods of modulating a signal to a cell mediated by IL-1δ or IL-1ε

25 comprising contacting the cell to an administered agonist or antagonist of IL-1R6. In some forms, the modulating is inhibiting, and the signal is a pro-inflammatory signal; or the antagonist is a neutralizing antibody to IL-1R6; the agonist or antagonist is administered in combination with an

30 antagonist or agonist of CXCR1, CXCR2, or CCR6; or the agonist or antagonist is administered in combination with a growth factor, cytokine, chemokine, or immune adjuvant. Certain combinations include contacting with another anti-inflammatory agent.

Other methods include selectively labeling a population
of cells, the method comprising contacting the cells with an
IL-1R6 antibody or a cytokine selected from IL-1δ or IL-1ε,
thereby resulting in the identification of cells expressing

5   IL-1R6. Preferably, the contacting results in modulation of
NFkB activation; the labeling allows purification of IL-1R6+
cells; or the labeling allows depletion of IL-1R6+ cells.
Such methods also alow preparation of a population of cells,
e.g., which: bind anti-IL-1R6 antibody or antiserum; or are

10   prepared by Fluorescent Activated Cell Sorting with a labeled
IL-1R6 selective: ligand; antibody; or binding compound
comprising the antigen binding portion from an antibody which
selectively binds IL-1R6.

The invention also provides means to test a compound for

15   ability to affect IL-1R6 receptor-ligand interaction, the
method comprising comparing the interaction of IL-1R6 with IL-
1δ or IL-1ε in the presence and absence of the compound. In
some forms, the compound is an antibody against IL-1R6, IL-1δ,
or IL-1ε.

20   Compositions are also provided based upon the seqeunces
provided herein, e.g., an isolated or recombinant
polynucleotide which: encodes at least 15 contiguous amino
acid residues of SEQ ID NO: 2; encodes at least two distinct
segments of at least 8 contiguous amino acid residues of SEQ

25   ID NO 2; comprises one or more segments at least 21 contiguous
nucleotides of SEQ ID NO: 1; encodes at least 15 contiguous
amino acid residues of SEQ ID NO: 4; encodes at least two
distinct segments of at least 8 contiguous amino acid residues
of SEQ ID NO 4; or comprises one or more segments at least 21

30   contiguous nucleotides of SEQ ID NO: 3. Polypeptide
embodiments are provided, e.g., an isolated or recombinant
antigenic polypeptide comprising at least: one segment of 12
identical contiguous amino acids from SEQ ID NO: 2; at least
two distinct segments of 8 identical contiguous amino acids

from SEQ ID NO: 2; one segment of 12 identical contiguous
amino acids from SEQ ID NO: 4; or at least two distinct
segments of 8 identical contiguous amino acids from SEQ ID NO:
4. Binding compositions are also provided, e.g., binding
compound comprising an antigen binding portion from an
antibody which binds with selectivity to the polypeptides.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

OUTLINE

I.   General
II.   Activities
III.   Nucleic acids
   A.   encoding fragments, sequence, probes
   B.   mutations, chimeras, fusions
   C.   making nucleic acids
   D.   vectors, cells comprising
IV.   Proteins, Peptides
   A.   fragments, sequence, immunogens, antigens
   B.   muteins
   C.   agonists/antagonists, functional equivalents
   D.   making proteins
V.   Making nucleic acids, proteins
VI.   Antibodies
   A.   polyclonals
   B.   monoclonal, Kd
   C.   anti-idiotypic antibodies
   D.   hybridoma cell lines
VII.   Kits and Methods to quantify IL-1δ
   A.   ELISA
   B.   assay mRNA encoding
   C.   qualitative/quantitative
   D.   kits
VIII.   Therapeutic compositions, methods

A.   combination compositions

B.   unit dose

C.   administration

IX.   Receptors

5

I.   General

Before the present compositions, formulations, and methods are described, it is to be understood that this invention is not limited to the particular methods, 10 compositions, and cell lines described herein, as such methods, compositions, and cell lines may, of course, vary.   It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments.

As used herein, including the appended claims, singular 15 forms of words such as "a," "an," and "the" include their corresponding plural referents unless the context clearly dictates otherwise.   Thus, e.g., reference to "an organism" includes one or more different organisms, reference to "a cell" includes one or more of such cells, and reference to "a 20 method" includes reference to equivalent steps and methods known to a person of ordinary skill in the art, and so forth.

Unless otherwise defined, technical and scientific terms used herein have the same meaning as commonly understood by a person of ordinary skill in the art to which this invention 25 belongs.   Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below.   Publications, patent applications, patents, and other references discussed above 30 are provided solely for their disclosure prior to the filing date of the present application.   Nothing herein is to be construed as an admission that the invention is not entitled to antedate any such disclosure by virtue of its prior invention.   Publications, patent applications, patents, and

other references mentioned herein are incorporated by reference in their entirety including all figures and drawings.

5   The present invention provides the amino acid sequence and DNA sequence of primate, e.g., human, interleukin-1 like molecule having particular defined properties, both structural and biological. These have been designated herein as interleukin-1δ (IL-1δ). Non-human primate counterparts should also exist, since rodents possess a counterpart. The nucleic

10   acids encompassed herein include DNA, cDNA, and RNA sequences which encode IL-1δ. It is understood that nucleic acids encoding all or a portion of the primate IL-1δ polypeptides are also encompassed. Such nucleic acids include both naturally occurring and intentionally manipulated nucleic

15   acids. For example, IL-1δ may be subjected to site-directed mutagenesis.

      IL-1 family members are known to alter the host response to an inflammatory, infectious or immunological challenge. Dinarello (1994) Eur. Cytokine Netw. 5:517-531. IL-1s are in

20   fact 'primary' cytokines as IL-1s are capable of inducing and orchestrating inflammation. IL-1 activity, therefore, is tightly controlled under physiological conditions. The classical IL-1 family comprises several ligands (e.g., IL-1α, IL-1β, and IL-1 receptor antagonist (IL-1RA) (March, et al.

25   (1985) Nature 315:641-647; Hannum, et al. (1990) Nature 343:336-340; and Eisenberg, et al. (1990) Nature 343:341-346), and surface and soluble IL-1 receptors (IL-1RI, RII, and IL-1R accessory proteins designated IL-1R1, 2, and 3 herein, respectively; see, Sims, et al. (1988) Science 241:585-589;

30   McMahan, et al. (1991) EMBO J. 10:2821-2832; and Greenfeder, et al. (1995) J. Biol. Chem. 270:13757-13765, in keeping with the previously proposed numbering system, as in Rock, et al. (1998) Proc. Nat'l Acad. Sci. USA 95:588-593). IL-1 signaling is initiated by high-affinity binding of IL-1α/β to IL-1R1,

which gets subsequently bound by R3 (Sims, et al. (1988)
Science 241:585-589; and Greenfeder, et al. (1995) J. Biol.
Chem. 270:13757-13765). This results in an intracellular
signaling cascade quite similar to stress-induced signal

5   transduction (Freshney, et al. (1994) Cell 78:1039-1049), with
the end effect being NFκB activation (O'Neill and Greene
(1998) J. Leukoc. Biol. 63:650-657). IL-1 signaling is
unusually efficient, as only one to ten IL-1 molecules bound
per cell are required to trigger an IL-1 response. Nature

10   designed specific 'roadblocks' to reduce the IL-1 response
when necessary. In addition to controlling gene expression,
synthesis and secretion, this regulation extends to
antagonists of the IL-1 response. IL-1RA and IL-1R2
antagonize the response to IL-1α/β at the receptor and ligand

15   level, respectively. Hannum, et al. (1990) Nature 343:336-
340; Eisenberg, et al. (1990) Nature 343:341-346; and Colotta,
et al. (1994) Immunol. Today 15:562-566). Numerous studies
have shown that perturbation of such control contributes to
the pathogenesis of inflammatory and immunological diseases

20   (e.g., leukemias, rheumatoid arthritis, and psoriasis).
Dinarello (1996) Blood 87:2095-2147.

        Recently, additional members were added to the IL-1
family based on sequence homology and the presence of key
structural patterns. For example, IL-18 (IL-1γ; Okamura, et

25   al. (1995) Nature 378:88-91; Ushio, et al. (1996) J. Immunol.
156:4274-4279) is predicted to fold as a β-rich trefoil,
typical for IL-1 ligands (Bazan, et al. (1996) Nature
379:591). Moreover, with respect to processing, receptor
usage and signaling, IL-18/IL-1γ can be classified as an IL-1

30   family member. Ghayur, et al. (1997) Nature 386:619-623; Gu,
et al. (1997) Science 275:206-209; Torigoe, et al. (1997) J.
Biol. Chem. 272:25737-25742; Born, et al. (1998) J. Biol.
Chem. 273:29445-29450; Robinson, et al. (1997) Immunity.
7:571-581; and Kojima, et al. (1998) Biochem. Biophys. Res.

Commun. 244:183-186. Also at the receptor level, additional
IL-1R-like molecules exist, many of which are currently orphan
receptors, such as T1/ST2 (termed IL-1R4; Klemenz, et al.
(1989) Proc. Nat'l Acad. Sci. USA 86:5708-5712; and Tominaga

5   (1989) FEBS Lett. 258:301-304), IL-1Rrp1 (IL-1R related
protein 1; IL-1R5; Parnet et al. (1996) J. Biol. Chem.
271:3967-3970) and IL-1R related protein 2 (IL-1R6; Lovenberg,
et al. (1996) J. Neuroimmunol. 70:113-122), IL-1RacPL (IL-1R7;
Born, et al. (1998) J. Biol. Chem. 273:29445-29450), single Ig

10   domain IL-1R-related protein (SIGIRR, e.g., IL-1R8; Thomassen,
et al. (1999) Cytokine 11:389-399), IL-1R accessory protein-
like (IL-1RAPL; IL-1R9; Carrie, et al. (1999) Nat. Genet.
23:25-31), and IL-1R10 (Sana, T.R., R. Debets, J.C. Timans,
J.F. Bazan, and R.A. Kastelein: submitted for publication),

15   all harboring extracellular Ig-folds and an intracellular
domain homologous to the cytosolic part of the Drosophila Toll
protein. It is interesting to note that the majority of the
IL-1 ligands (e.g., IL-1α/β/ra) and IL-1Rs (e.g., IL-1R1, 2,
4, 5, 6, and 7) are clustered and localized to Chr. 2 (Born,

20   et al. (1998) J. Biol. Chem. 273:29445-29450; Lafage, et al.
(1989) Blood 73:104-107; Patterson, et al. (1993) Genomics
15:173-176; Sims, et al. (1995) Cytokine 7:483-490; and Dale
and Nicklin (1999) Genomics 57:177-179.
       The availability of several orphan IL-1Rs points to the

25   existence of other IL-1 ligands. Two novel IL-1 ligands were
identified, based on homology with IL-1RA, which have been
designated IL-1δ and IL-1ε. These novel IL-1s are strongly
expressed in embryonic tissue and epithelial cells, such as
skin keratinocytes. Adenovirally-derived human IL-1δ and IL-1ε

30   proteins do not activate NFκB through the classical IL-1Rs,
e.g., the IL-1Rs used by IL-1α/β or IL-18. Instead, IL-1ε
activates this transcription factor via IL-1R6 and this
response is potently and specifically antagonized by IL-1δ.

The expression of IL-1ε, and to a lesser extent of
IL-1δ, is significantly upregulated in IL-1β/TNFα-stimulated
human keratinocytes. Human IL-1δ and IL-1ε proteins do not
activate NFκB through the classical IL-1Rs, i.e., the IL-1Rs

5   used by IL-1α/β (IL-1R1 and IL-1R3) or IL-18 (IL-1R5/IL-1R7).
Instead, IL-1ε activates this transcription factor via IL-1R6
and this response is potently and specifically antagonized by
IL-1δ. Lesional psoriasis skin shows a substantially increased
expression of both the IL-1 ligands as well as their IL-1R.

10   IL-1δ/ε and IL-1R6 probably constitute an independent signaling
system, present in epithelial barriers of the body, which may
take part in local inflammatory responses.

Some of the standard methods applicable are described or
referenced, e.g., in Maniatis, et al. (1982) Molecular

15   Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory,
Cold Spring Harbor Press; Sambrook, et al. (1989) Molecular
Cloning: A Laboratory Manual, (2d ed.), vols 1-3, CSH Press,
NY; Ausubel, et al., Biology, Greene Publishing Associates,
Brooklyn, NY; or Ausubel, et al. (1987 and periodic

20   supplements) Current Protocols in Molecular Biology,
Greene/Wiley, New York; each of which is incorporated herein
by reference. See also USSN 09/130,972, which is incorporated
herein by reference.

A complete nucleotide and corresponding amino acid

25   sequence of a primate, e.g., human, IL-1δ coding segment is
shown in SEQ ID NO: 1 and 2. Nucleic and amino acid sequences
of IL-1ε are shown in SEQ ID NO:3, 4, and 10 (human and
rodent. Figure 1 shows an alignment of selected family
members.

30   For the primate, e.g., human, IL-1δ, the beta sheet
regions run from about leu7-met11; val18-leu21; leu26-gly29;
ile42-asn47; pro56-val61; gln66-gly71; thr77-pro82; phe98-
arg103; ser110-ser113; trp119-thr123; pro130-thr134; and

pro145-gln153. Site A residues correspond to about lys12;
ser14-leu16; leu21-asn23; leu27; gly29-gly34; val36; lys38;
pro125-glu126; asp128-pro130; tyr150, and gln152. Site B
residues correspond to about met1-gly5; leu7; asn47; trp49;
5 ser53; ser55; gly91-lys93; arg102; asp104-met105; leu107-
thr108; gln153; and asp155. Asp148 corresponds to a residue
which is particularly important in the IL-1RA.

For the primate, e.g., human, IL-1ε, the beta sheet
regions run from about ile23-ile27; val34-thr36; leu42-val45;
10 val56-cys61; pro74-ile79; met84-glu89; thr96-glu101; phe117-
ala122; thr129-ser132; trp138-ser142; pro148-thr152; and
tyr159-ile167. Site A residues correspond to about asn28;
leu30-gln32; thr36-gln38; val43; val45-ser50; thr52; lys144-
arg145; gln147-pro148; glu164; and asn166. Site B residues
15 correspond to about gln17-cys20; ile23; cys61; tyr63; glu68;
gly70-arg71; asp73; gln110-glu112; arg121; lys123-thr124;
arg126-thr127; ile167; and asp169. Ala162 corresponds to a
residue which is particularly important in the IL-1RA.

Segments with boundaries adjacent these positions will be
20 particularly useful. Mutagenesis in these regions will be used
to determine structure-activity relationship, particularly with
the receptor, as provided herein.

Complete nucleotide and corresponding amino acid sequences
of mammalian, e.g., primate and rodent, e.g., human and mouse,
25 IL-1R6 coding segments are shown SEQ ID Nos:12, 13, 14, and 15.

As used herein, the term "primate IL-1δ" shall be used to
describe a protein comprising a protein or peptide segment
having or sharing the amino acid sequence shown in SEQ ID
NO:2, or a substantial fragment thereof; but distinct from
30 rodent sequences. The invention also includes protein
variations of the IL-1δ allele whose sequence is provided,
e.g., a mutein agonist or antagonist. Typically, such
agonists or antagonists will exhibit less than about 10%
sequence differences, and thus will often have between 1- and

11-fold substitutions, e.g., 2-, 3-, 5-, 7-fold, and others.
It also encompasses allelic and other variants, e.g., natural
polymorphic variants, of the protein described. "Natural" as
used herein means unmodified by artifice, found, e.g., in
5   natural sources. Typically, it will bind to its corresponding
biological receptor with high affinity, e.g., at least about
100 nM, usually better than about 30 nM, preferably better
than about 10 nM, and more preferably at better than about 3
nM. The term shall also be used herein to refer to related
10   naturally occurring forms, e.g., alleles, polymorphic
variants, and metabolic variants of the primate protein.

     This invention also encompasses proteins or peptides
having substantial amino acid sequence homology with the amino
acid sequence in SEQ ID NO:2. It will include sequence
15   variants with relatively few substitutions, e.g., preferably
less than about 3-5.

     A substantial polypeptide "fragment", or "segment", is a
stretch of amino acid residues of at least about 8 amino
acids, generally at least 10 amino acids, more generally at
20   least 12 amino acids, often at least 14 amino acids, more
often at least 16 amino acids, typically at least 18 amino
acids, more typically at least 20 amino acids, usually at
least 22 amino acids, more usually at least 24 amino acids,
preferably at least 26 amino acids, more preferably at least
25   28 amino acids, and, in particularly preferred embodiments, at
least about 30 or more amino acids. Sequences of segments of
different proteins can be compared to one another over
appropriate length stretches.

     Amino acid sequence homology, or sequence identity, is
30   determined by optimizing residue matches, if necessary, by
introducing gaps as required. See, e.g., Needleham, et al.,
(1970) J. Mol. Biol. 48:443-453; Sankoff, et al., (1983)
chapter one in Time Warps, String Edits, and Macromolecules:
The Theory and Practice of Sequence Comparison, Addison-

Wesley, Reading, MA; and software packages from
IntelliGenetics, Mountain View, CA; and the University of
Wisconsin Genetics Computer Group (GCG), Madison, WI; each of
which is incorporated herein by reference. This changes when
5   considering conservative substitutions as matches.
Conservative substitutions typically include substitutions
within the following groups: glycine, alanine; valine,
isoleucine, leucine; aspartic acid, glutamic acid; asparagine,
glutamine; serine, threonine; lysine, arginine; and
10 phenylalanine, tyrosine. Homologous amino acid sequences are
intended to include natural allelic and interspecies
variations in the cytokine sequence. Typical homologous
proteins or peptides will have from 50-100% homology (if gaps
can be introduced), to 60-100% homology (if conservative
15 substitutions are included) with an amino acid sequence
segments of SEQ ID NO:2 or 4. Homology measures will be at
least about 70%, generally at least 76%, more generally at
least 81%, often at least 85%, more often at least 88%,
typically at least 90%, more typically at least 92%, usually
20 at least 94%, more usually at least 95%, preferably at least
96%, and more preferably at least 97%, and in particularly
preferred embodiments, at least 98% or more. The degree of
homology will vary with the length of the compared segments.
Homologous proteins or peptides, such as the allelic variants,
25 will share most biological activities with the embodiments
described in SEQ ID NO:2. As used herein, the term
"biological activity" is used to describe, without limitation,
effects on inflammatory responses and/or innate immunity. For
example, they may, like IL-1γ, exhibit synergistic induction by
30 splenocytes of IFN-γ in combination with IL-12 or IL-2, with or
without anti-type I or anti-type II IL-1 receptor antibodies,
or more structural properties as receptor binding and
cross-reactivity with antibodies raised against the same or a
polymorphic variant of a mammalian IL-1δ.

The terms ligand, agonist, antagonist, and analog of IL-1δ include molecules that modulate the characteristic cellular responses to IL-1δ or IL-1δ-like proteins, as well as molecules possessing the more standard structural binding competition features of ligand-receptor interactions, e.g., where the receptor is a natural receptor or an antibody. The cellular responses likely are mediated through binding of IL-1δ to cellular receptors related to, but possibly distinct from, the type I or type II IL-1 receptors. Also, a ligand is a molecule which serves either as a natural ligand to which said receptor, or an analog thereof, binds, or a molecule which is a functional analog of the natural ligand. The functional analog may be a ligand with structural modifications, or may be a wholly unrelated molecule which has a molecular shape which interacts with the appropriate ligand binding determinants. The ligands may serve as agonists or antagonists, see, e.g., Goodman, et al. (eds.) (1990) Goodman & Gilman's: The Pharmacological Bases of Therapeutics, Pergamon Press, New York.

Rational drug design may also be based upon structural studies of the molecular shapes of a receptor or antibody and other effectors or ligands. Effectors may be other proteins which mediate other functions in response to ligand binding, or other proteins which normally interact with the receptor. One means for determining which sites interact with specific other proteins is a physical structure determination, e.g., x-ray crystallography or 2 dimensional NMR techniques. These will provide guidance as to which amino acid residues form molecular contact regions. For a detailed description of protein structural determination, see, e.g., Blundell and Johnson (1976) Protein Crystallography, Academic Press, New York, which is hereby incorporated herein by reference.

II. Activities

The IL-1δ proteins will have a number of different
biological activities, e.g., in the immune system, and will
include inflammatory functions or disorders, e.g., psoriasis,
or other innate immunity responses. The IL-1δ or IL-1ε
5   proteins are homologous to other IL-1 proteins, but each have
structural differences. For example, a human IL-1γ gene coding
sequence probably has about 70% identity with the nucleotide
coding sequence of mouse IL-1γ, and similar measures of
similarity will apply to the IL-1δ and IL-1ε. At the amino
10   acid level, there is also likely to be about 60% identity.
This level of similarity suggests that the new IL-1δ and IL-1ε
proteins are related to the other IL-1α and IL-1β and IL-1RA.

The mouse IL-1γ molecule has the ability to stimulate IFN-
γ production which augments NK activity in spleen cells. See
15   Okamura, et al. (1995) Nature 378:88-91.

The activities of the mouse IL-1α, IL-1β, and IL-1γ have
been compared as to their ability to induce IFN-γ, alone or in
combination with IL-2 or IL-12 in SCID splenocytes and
purified NK cells. See Hunter, et al. (1995) J. Immunol.
20   155:4347-4354; and Bancroft, et al. (1991) Immunol. Revs.
124:5-24. The IL-1γ was found to be much more potent in
stimulating IFN-1γ than either IL-1α or IL-1β. IL-1δ and IL-1ε
and their agonists or antagonists should have related
activities, typically affecting similar immune functions,
25   including inflammatory responses.

In IL-2 activated NK cells, IFN-γ production is blocked by
the addition of anti-IL-1β antibodies. See Hunter, et al.
(1995). However, mouse IL-1γ can overcome this block and
induce IFN-γ. This is the only cytokine known to be able to do
30   this. In addition, in vivo, administration of mouse IL-1γ to
mice infected with the parasite T. Cruzi significantly
decreases parasitemia. IL-1δ and IL-1ε and their agonists or

antagonists should operate through related mechanisms and effectors.

The present disclosure also describes new assays for activities predicted for the IL-1δ and IL-1ε molecules.

5 Corresponding activities should be found in other mammalian systems, including primates. It is likely that the new IL-1-like molecules produced by similar recombinant means to the human IL-1γ protein should exhibit a biological activity of modulating lymphocyte cells in production of IFN-γ. See assays

10 described, e.g., in de Waal Malefyt, et al., in de Vries and de Waal Malefyt (eds. 1995) "Interleukin-10" Landes Co., Austin, TX. Furthermore, there is substantial likelihood of synergy with other IL-1 or IL-12 related agonists or antagonists. It is likely that the receptors, which are

15 expected to include multiple different polypeptide chains, exhibit species specificity for their corresponding ligands. The IL-1α and IL-1β ligands both signal through heterodimeric receptors.

20 III. Nucleic Acids

This invention contemplates use of isolated nucleic acid or fragments, e.g., which encode this or a closely related protein, or fragments thereof, e.g., to encode a biologically active corresponding polypeptide.

25 The term "isolated nucleic acid or fragments" as used herein means a nucleic acid, e.g., a DNA or RNA molecule, that is not immediately contiguous with sequences present in the naturally occurring genome of the organism from which it is derived. Thus, the term describes, e.g., a nucleic acid that

30 is incorporated into a vector, such as a plasmid or viral vector; a nucleic acid that is incorporated into the genome of a heterologous cell (or the genome of homologous cell), but at a site different from that at which it normally occurs); and a nucleic acid that exists as a separate molecule, e.g., a DNA

fragment produced by PCR amplification or restriction enzyme
digestion, or an RNA molecule produced by in vitro
transcription. The term also describes a recombinant (e.g.,
genetically engineered) nucleic acid that forms part of a
5   hybrid gene encoding additional polypeptide sequences that can
be used, e.g., in the production of a fusion protein. In
addition, this invention embodies any engineered or nucleic
acid molecule created by artifice that encodes a biologically
active protein or polypeptide having characteristic IL-1δ or
10 IL-1ε activity. Typically, the nucleic acid is capable of
hybridizing, under appropriate conditions, with a nucleic acid
sequence segment shown in SEQ ID NO:1. Further, this
invention covers the use of isolated or recombinant nucleic
acid, or fragments thereof, which encode proteins having
15 fragments which are homologous to the newly disclosed IL-1-
like proteins. The isolated nucleic acids can have the
respective regulatory sequences in the 5' and 3' flanks, e.g.,
promoters, enhancers, poly-A addition signals, and others from
the natural gene.
20   An "isolated" nucleic acid is a nucleic acid, e.g., an
RNA, DNA, or a mixed polymer, which is substantially pure,
e.g., separated from other components which naturally
accompany a native sequence, such as ribosomes, polymerases,
and flanking genomic sequences from the originating species.
25 The term embraces a nucleic acid sequence which has been
removed from its naturally occurring environment, and includes
recombinant or cloned DNA isolates, which are thereby
distinguishable from naturally occurring compositions, and
chemically synthesized analogs or analogs biologically
30 synthesized by heterologous systems. A substantially pure
molecule includes isolated forms of the molecule, either
completely or substantially pure.

     An isolated nucleic acid will generally be a homogeneous
composition of molecules, but will, in some embodiments,

contain heterogeneity, preferably minor. This heterogeneity
is typically found at the polymer ends or portions not
critical to a desired biological function or activity.

5   A "recombinant" nucleic acid is defined either by its
method of production or its structure. In reference to its
method of production, e.g., a product made by a process, the
process is use of recombinant nucleic acid techniques, e.g.,
involving human intervention in the nucleotide sequence.
Typically this intervention involves in vitro manipulation,
10   although under certain circumstances it may involve more
classical animal breeding techniques. Alternatively, it can
be a nucleic acid made by generating a sequence comprising
fusion of two fragments which are not naturally contiguous to
each other, but is meant to exclude products of nature, e.g.,
15   naturally occurring mutants as found in their natural state.
Thus, e.g., products made by transforming cells with any
unnaturally occurring vector is encompassed, as are nucleic
acids comprising sequence derived using any synthetic
oligonucleotide process. Such a process is often done to
20   replace a codon with a redundant codon encoding the same or a
conservative amino acid, while typically introducing or
removing a restriction enzyme sequence recognition site.
Alternatively, the process is performed to join together
nucleic acid segments of desired functions to generate a
25   single genetic entity comprising a desired combination of
functions not found in the commonly available natural forms,
e.g., encoding a fusion protein. Restriction enzyme
recognition sites are often the target of such artificial
manipulations, but other site specific targets, e.g.,
30   promoters, DNA replication sites, regulation sequences,
control sequences, or other useful features may be
incorporated by design. A similar concept is intended for a
recombinant, e.g., fusion, polypeptide. This will include a
dimeric repeat. Specifically included are synthetic nucleic

acids which, by genetic code redundancy, encode similar
polypeptides to fragments of the IL-1δ or IL-1ε and fusions of
sequences from various different interleukin or related
molecules, e.g., growth factors.

5   A "fragment" in a nucleic acid context is a contiguous
segment of at least about 17 nucleotides, generally at least
21 nucleotides, more generally at least 25 nucleotides,
ordinarily at least 30 nucleotides, more ordinarily at least
35 nucleotides, often at least 39 nucleotides, more often at

10   least 45 nucleotides, typically at least 50 nucleotides, more
typically at least 55 nucleotides, usually at least 60
nucleotides, more usually at least 66 nucleotides, preferably
at least 72 nucleotides, more preferably at least 79
nucleotides, and in particularly preferred embodiments will be

15   at least 85 or more nucleotides including, e.g., 100, 150,
200, 250, etc. Typically, fragments of different genetic
sequences can be compared to one another over appropriate
length stretches, particularly defined segments such as the
domains described below.

20   . A nucleic acid which codes for an IL-1δ will be
particularly useful to identify genes, mRNA, and cDNA species
which code for itself or closely related proteins, as well as
DNAs which code for polymorphic, allelic, or other genetic
variants, e.g., from different individuals or related species.

25   Preferred probes for such screens are those regions of the
interleukin which are conserved between different polymorphic
variants or which contain nucleotides which lack specificity,
and will preferably be full length or nearly so. In other
situations, polymorphic variant specific sequences will be

30   more useful.

      This invention further covers recombinant nucleic acid
molecules and fragments having a nucleic acid sequence
identical to or highly homologous to the isolated DNA set
forth herein. In particular, the sequences will often be

operably linked to DNA segments which control transcription, translation, and DNA replication. These additional segments typically assist in expression of the desired nucleic acid segment.

5   Homologous nucleic acid sequences, when compared to one another or SEQ ID NO:1 sequences, exhibit significant similarity. The standards for homology in nucleic acids are either measures for homology generally used in the art by sequence comparison or based upon hybridization conditions.

10 Comparative hybridization conditions are described in greater detail below.

Substantial identity in the nucleic acid sequence comparison context means either that the segments, or their complementary strands, when compared, are identical when

15 optimally aligned, with appropriate nucleotide insertions or deletions, in at least about 60% of the nucleotides, generally at least 66%, ordinarily at least 71%, often at least 76%, more often at least 80%, usually at least 84%, more usually at least 88%, typically at least 91%, more typically at

20 about 93%, preferably at least about 95%, more preferably at least about 96 to 98% or more, and in particular embodiments, as high at about 99% or more of the nucleotides, including, e.g., segments encoding structural domains such as the segments described below. Alternatively, substantial identity

25 will exist when the segments will hybridize under selective hybridization conditions, to a strand or its complement, typically using a sequence derived from SEQ ID NO:1 or 3. Typically, selective hybridization will occur when there is at least about 55% homology over a stretch of at least about 14

30 nucleotides, more typically at least about 65%, preferably at least about 75%, and more preferably at least about 90%. See, Kanehisa (1984) Nuc. Acids Res. 12:203-213. The length of homology comparison, as described, may be over longer stretches, and in certain embodiments will be over a stretch

of at least about 17 nucleotides, generally at least about 20
nucleotides, ordinarily at least about 24 nucleotides, usually
at least about 28 nucleotides, typically at least about 32
nucleotides, more typically at least about 40 nucleotides,
5   preferably at least about 50 nucleotides, and more preferably
at least about 75 to 100 or more nucleotides.

Stringent conditions, in referring to homology in the
hybridization context, will be stringent combined conditions
of salt, temperature, organic solvents, and other parameters
10   typically controlled in hybridization reactions. Stringent
temperature conditions will usually include temperatures in
excess of about 30° C, more usually in excess of about 37° C,
typically in excess of about 45° C, more typically in excess
of about 55° C, preferably in excess of about 65° C, and more
15   preferably in excess of about 70° C. Stringent salt
conditions will ordinarily be less than about 500 mM, usually
less than about 400 mM, more usually less than about 300 mM,
typically less than about 200 mM, preferably less than about
100 mM, and more preferably less than about 80 mM, even down
20   to less than about 20 mM. Certain detergents or destabilizing
reagents may be added, e.g., formamide at 50%, etc. However,
the combination of parameters is much more important than the
measure of any single parameter. See, e.g., Wetmur and
Davidson (1968) J. Mol. Biol. 31:349-370, which is hereby
25   incorporated herein by reference.

The isolated DNA can be readily modified by nucleotide
substitutions, nucleotide deletions, nucleotide insertions,
and inversions of nucleotide stretches. These modifications
result in novel DNA sequences which encode this protein or its
30   derivatives. These modified sequences can be used to produce
mutant proteins (muteins) or to enhance the expression of
variant species. Enhanced expression may involve gene
amplification, increased transcription, increased translation,
and other mechanisms. Such mutant IL-1—like derivatives

include predetermined or site-specific mutations of the
protein or its fragments, including silent mutations using
genetic code degeneracy. "Mutant IL-1δ" as used herein
encompasses a polypeptide otherwise falling within the
5   homology definition of the IL-1δ as set forth above, but
having an amino acid sequence which differs from that of other
IL-1-like proteins as found in nature, whether by way of
deletion, substitution, or insertion. In particular, "site
specific mutant IL-1δ" encompasses a protein having
10 substantial homology with a protein of SEQ ID NO:2, and
typically shares most of the biological activities of the form
disclosed herein.

     Although site specific mutation sites are predetermined,
mutants need not be site specific. Mammalian IL-1δ
15 mutagenesis can be achieved by making amino acid insertions or
deletions in the gene, coupled with expression.
Substitutions, deletions, insertions, or any combinations may
be generated to arrive at a final construct. Insertions
include amino- or carboxy- terminal fusions. Random
20 mutagenesis can be conducted at a target codon and the
expressed mammalian IL-1δ mutants can then be screened for the
desired activity. Methods for making substitution mutations
at predetermined sites in DNA having a known sequence are well
known in the art, e.g., by M13 primer mutagenesis. See also
25 Sambrook, et al. (1989) and Ausubel, et al. (1987 and periodic
Supplements).

     The mutations in the DNA normally should not place coding
sequences out of reading frames and preferably will not create
complementary regions that could hybridize to produce
30 secondary mRNA structure such as loops or hairpins.

     The phosphoramidite method described by Beaucage and
Carruthers (1981) <u>Tetra. Letts.</u> 22:1859-1862, will produce
suitable synthetic DNA fragments. A double stranded fragment
will often be obtained either by synthesizing the

complementary strand and annealing the strand together under appropriate conditions or by adding the complementary strand using DNA polymerase with an appropriate primer sequence.

5   Polymerase chain reaction (PCR) techniques can often be applied in mutagenesis. Alternatively, mutagenesis primers are commonly used methods for generating defined mutations at predetermined sites. See, e.g., Innis, et al. (eds. 1990) <u>PCR Protocols: A Guide to Methods and Applications</u> Academic Press, San Diego, CA; and Dieffenbach and Dveksler (eds. 1995) <u>PCR</u>

10   <u>Primer: A Laboratory Manual</u> Cold Spring Harbor Press, CSH, NY.

IV.   Proteins, Peptides

As described above, the present invention encompasses
15   primate IL-1δ, e.g., whose sequences are disclosed in SEQ ID NO:2, and described above. Allelic and other variants are also contemplated, including, e.g., fusion proteins combining portions of such sequences with others, including epitope tags and functional domains.

20   The present invention also provides recombinant proteins, e.g., heterologous fusion proteins using segments from these rodent proteins. A heterologous fusion protein is a fusion of proteins or segments which are naturally not normally fused in the same manner. Thus, the fusion product of a growth factor
25   with an interleukin is a continuous protein molecule having sequences fused in a typical peptide linkage, typically made as a single translation product and exhibiting properties derived from each source peptide. A similar concept applies to heterologous nucleic acid sequences.

30   In addition, new constructs may be made from combining similar functional or structural domains from other related proteins, e.g., growth factors or other cytokines. For example, receptor-binding or other segments may be "swapped" between different new fusion polypeptides or fragments. See,

e.g., Cunningham, et al. (1989) Science 243:1330-1336; and
O'Dowd, et al. (1988) J. Biol. Chem. 263:15985-15992, each of
which is incorporated herein by reference. Thus, new chimeric
polypeptides exhibiting new combinations of specificities will
5   result from the functional linkage of receptor-binding
specificities. For example, the receptor binding domains from
other related ligand molecules may be added or substituted for
other domains of this or related proteins. The resulting
protein will often have hybrid function and properties. For
10   example, a fusion protein may include a targeting domain which
may serve to provide sequestering of the fusion protein to a
particular organ, e.g., a ligand portions which is
specifically bound by spleen cells and would serve to
accumulate in the spleen.

15   Candidate fusion partners and sequences can be selected
from various sequence data bases, e.g., GenBank, c/o
IntelliGenetics, Mountain View, CA; and BCG, University of
Wisconsin Biotechnology Computing Group, Madison, WI, which
are each incorporated herein by reference.

20   The present invention particularly provides muteins which
act as agonists or antagonists of the IL-1δ. Structural
alignment of IL-1δ and IL-1ε with other members of the IL-1
family show conserved features/residues, particularly 12 β
strands folded into a β-trefoil fold.

25   Alignment of the IL-1δ and IL-1ε sequences with other
members of the IL-1 family indicates that the β conformations
correspond to similar sequences in other IL-1 family members.
See Figure 1 and, e.g., Bazan, et al. (1996) Nature 379:591;
Lodi, et al. (1994) Science 263:1762-1766; Sayle and Milner-
30   White (1995) TIBS 20:374-376; and Gronenberg, et al. (1991)
Protein Engineering 4:263-269.

The IL-1α and IL-1β ligands bind an IL-1 receptor type I
as the primary receptor and this complex then forms a high
affinity receptor complex with the IL-1 receptor type III.

Such receptor subunits are probably shared with the new IL-1
family members.

    The mouse IL-1γ does not bind to the known mouse IL-1
receptor types I, II (decoy receptor), or III. In addition,
5   the mouse IGIF biological activity cannot be blocked with
anti-type I, II, or III antibodies. This suggests that the
related mouse IGIF binds to receptors related to the IL-1
receptors already isolated, e.g., IL-1R related proteins 1
(IL-1Rrp1) and IL-RAcPL.

10   The solved structures for IL-1β, the natural IL-1
receptor antagonist (IL-1Ra), and a co-structure of IL-1Ra/IL-
1 receptor type I, however, suggest how to make an IL-1δ or
IL-1ε antagonist. See, e.g., accession numbers: U65590,
gbU19844, gbU19845, gi2173679, gi2170133, gi2172939, gbM15300,
15 gbM28983, gbU65590, gbM74294, embX04964, gi2169698, gi2169368
emb270047, gi914939, gi220782, embX52731, embX56972 and
embX12497, for various species examples of IL-1 family
members. Structural analyses of the mature IL-1δ or IL-1ε
suggest that its β-trefoil structures contact the IL-1
20 receptor over three binding sites (designated A, B, and C).
Sites A and C bind to the first receptor subunit (alpha) of
IL-1 while site B binds the IL-1 second receptor subunit
(beta). Homology sequence comparison of the IL-1 family
members reveals that the only known antagonist to IL-1
25 receptor (IL-1ra; Figure 1) is missing an amino acid domain
bounded by the β4 and β5 strands. This domain maps to a
portion of site B in rodent IL-1δ or IL-1ε (Figure 1) that
binds to the IL-1 second receptor subunit, suggesting that its
absence confers antagonist activity as evidenced by homology
30 comparison among other IL-1 family members. This loop portion
of contact site B spans approximately 7-10 amino acid
residues, while in IL-1RA the loop is "cut off" with only 2
residues remaining. Therefore, IL-1RA binds normally to

receptor type I, but cannot interact with receptor type III.
This makes IL-1RA into an effective IL-1 antagonist.

The corresponding location in rodent IL-1δ or IL-1ε
(between β4 and β5) defines a domain that forms a polypeptide
5   loop which is part of a primary binding segment to the IL-1
receptor type (site B). With mouse IL-1RA, it was shown that
replacement of the mouse IL-1RA residues with those mouse IL-
1β residues introduced IL-1 activity to the IL-1RA variant (IL-
1RA could then bind type III receptor). Similar substitutions
10 should establish that type III receptor can probably be used
by mouse IL-1δ or IL-1ε proteins or muteins.

Sites A and C mediate binding of IL-1δ or IL-1ε to the
first IL-1 receptor subunit, e.g., an alpha receptor subunit.
Site A contacts correspond in primate IL-1δ to amino acid
15 residues 12, 14-16, 21-23, 27, 29-34, 36, 38, 125-126, 128-
130; 150, and 152 of SEQ ID NO: 2; while site C contacts
correspond in primate IL-1δ to amino acid residues 73-97 of
SEQ ID NO: 2. Site B contacts are defined in primate IL-1δ by
amino acid residues 1-5, 7, 47, 49, 53, 55, 91-93, 102, 104-
20 105, 107-108, 153, and 155. Site A contacts correspond in
primate IL-1ε to amino acid residues 28, 30-32, 36-38, 43, 45-
50, 52, 144-145, 147-148, 164, and 166 of SEQ ID NO: 13; while
site C contacts correspond in primate IL-1ε to amino acid
residues 73-97 of SEQ ID NO: 4. Site B contacts are defined
25 in primate IL-1ε by amino acid residues 17-20, 23, 61, 63, 68,
70-71, 73, 110-112, 121, 123-124, 126-127, 167, and 169 of SEQ
ID NO: 4.

Similar variations in other species counterparts of IL-1δ
ligand sequence, e.g., in the corresponding regions, should
30 provide similar interactions with receptor. Substitutions
with either mouse sequences or human sequences are indicated.
Conversely, conservative substitutions away from the receptor

binding interaction regions will probably preserve most
biological activities.

"Derivatives" of the mammalian IL-1δ include amino acid
sequence mutants, glycosylation variants, metabolic
5   derivatives and covalent or aggregative conjugates with other
chemical moieties. Covalent derivatives can be prepared by
linkage of functionality's to groups which are found in the
IL-1δ amino acid side chains or at the N- or C- termini, e.g.,
by means which are well known in the art. These derivatives
10   can include, without limitation, aliphatic esters or amides of
the carboxyl terminus, or of residues containing carboxyl side
chains, O-acyl derivatives of hydroxyl group-containing
residues, and N-acyl derivatives of the amino terminal amino
acid or amino-group containing residues, e.g., lysine or
15   arginine. Acyl groups are selected from the group of
alkyl-moieties including C3 to C18 normal alkyl, thereby
forming alkanoyl aroyl species.

In particular, glycosylation alterations are included,
e.g., made by modifying the glycosylation patterns of a
20   polypeptide during its synthesis and processing, or in further
processing steps. Particularly preferred means for
accomplishing this are by exposing the polypeptide to
glycosylating enzymes derived from cells which normally
provide such processing, e.g., mammalian glycosylation
25   enzymes. Deglycosylation enzymes are also contemplated. Also
embraced are versions of the same primary amino acid sequence
which have other minor modifications, including phosphorylated
amino acid residues, e.g., phosphotyrosine, phosphoserine, or
phosphothreonine.

30   A major group of derivatives are covalent conjugates of
the interleukin or fragments thereof with other proteins of
polypeptides. These derivatives can be synthesized in
recombinant culture such as N- or C-terminal fusions or by the
use of agents known in the art for their usefulness in

cross-linking proteins through reactive side groups.
Preferred derivatization sites with cross-linking agents are
at free amino groups, carbohydrate moieties, and cysteine
residues.

5   Fusion polypeptides between the interleukin and other
homologous or heterologous proteins are also provided.
Homologous polypeptides may be fusions between different
growth factors, resulting in, for instance, a hybrid protein
exhibiting ligand specificity for multiple different

10   receptors, or a ligand which may have broadened or weakened
specificity of binding to its receptor. Likewise,
heterologous fusions may be constructed which would exhibit a
combination of properties or activities of the derivative
proteins. Typical examples are fusions of a reporter

15   polypeptide, e.g., luciferase, with a segment or domain of a
receptor, e.g., a ligand-binding segment, so that the presence
or location of a desired ligand may be easily determined.
See, e.g., Dull, et al., U.S. Patent No. 4,859,609, which is
hereby incorporated herein by reference. Other gene fusion

20   partners include glutathione-S-transferase (GST), bacterial ß-
galactosidase, trpE, Protein A, ß-lactamase, alpha amylase,
alcohol dehydrogenase, yeast alpha mating factor, or other
cytokines, e.g., IL-1 family members. See, e.g., Godowski, et
al. (1988) Science 241:812-816.

25   The phosphoramidite method described by Beaucage and
Carruthers (1981) Tetra. Letts. 22:1859-1862, will produce
suitable synthetic DNA fragments. A double stranded fragment
will often be obtained either by synthesizing the
complementary strand and annealing the strand together under

30   appropriate conditions or by adding the complementary strand
using DNA polymerase with an appropriate primer sequence.

        Such polypeptides may also have amino acid residues which
have been chemically modified by phosphorylation, sulfonation,
biotinylation, or the addition or removal of other moieties,

particularly those which have molecular shapes similar to
phosphate groups. In some embodiments, the modifications will
be useful labeling reagents, or serve as purification targets,
e.g., affinity ligands.

5   Fusion proteins will typically be made by either
recombinant nucleic acid methods or by synthetic polypeptide
methods. Techniques for nucleic acid manipulation and
expression are described generally, e.g., in Sambrook, et al.
(1989) Molecular Cloning: A Laboratory Manual (2d ed.), Vols.
10 1-3, Cold Spring Harbor Laboratory, and Ausubel, et al. (eds.
1987 and periodic supplements) Current Protocols in Molecular
Biology, Greene/Wiley, New York, which are each incorporated
herein by reference. Techniques for synthesis of polypeptides
are described, e.g., in Merrifield (1963) J. Amer. Chem. Soc.
15 85:2149-2156; Merrifield (1986) Science 232: 341-347; and
Atherton, et al. (1989) Solid Phase Peptide Synthesis: A
Practical Approach, IRL Press, Oxford; each of which is
incorporated herein by reference. See also Dawson, et al.
(1994) Science 266:776-779 for methods to make larger
20 polypeptides.

        In another embodiment, the present invention relates to
substantially purified peptide fragments of IL-1δ or IL-1ε that
block binding between IL-1 family members and a target
receptor. Such peptide fragments could represent research and
25 diagnostic tools in the study of inflammatory reactions to
antigenic challenge and the development of more effective
anti-inflammatory therapeutics. In addition, pharmaceutical
compositions comprising isolated and purified peptide
fragments of IL-1δ or IL-1ε may represent effective anti-
30 inflammatory therapeutics.

        The term "substantially purified" as used herein refers
to a molecule, such as a peptide that is substantially free of
other proteins, lipids, carbohydrates, nucleic acids, or other
biological materials with which it is naturally associated.

For example, a substantially pure molecule, such as a
polypeptide, can be at least 60%, by dry weight, the molecule
of interest. One skilled in the art can purify IL-1δ or IL-1ε
peptides using standard protein purification methods and the
5   purity of the polypeptides can be determined using standard
methods including, e.g., polyacrylamide gel electrophoresis
(e.g., SDS-PAGE), column chromatography (e.g., high
performance liquid chromatography (HPLC)), and amino-terminal
amino acid sequence analysis.

10   The invention relates not only to fragments of naturally-
occurring IL-1δ or IL-1ε, but also to IL-1δ or IL-1ε mutants
and chemically synthesized derivatives of IL-1δ or IL-1ε that
block binding between IL-1 family members and a target
receptor.

15   For example, changes in the amino acid sequence of IL-1δ
or IL-1ε are contemplated in the present invention. IL-1δ or
IL-1ε can be altered by changing the nucleic acid sequence
encoding the protein. Preferably, only conservative amino
acid alterations are undertaken, using amino acids that have
20   the same or similar properties. Illustrative amino acid
substitutions include the changes of: alanine to serine;
arginine to lysine; asparagine to glutamine or histidine;
aspartate to glutamate; cysteine to serine; glutamine to
asparagine; glutamate to aspartate; glycine to proline;
25   histidine to asparagine or glutamine; isoleucine to leucine or
valine; leucine to valine or isoleucine; lysine to arginine,
glutamine, or glutamate; methionine to leucine or isoleucine;
phenylalanine to tyrosine, leucine or methionine; serine to
threonine; threonine to serine; tryptophan to tyrosine;
30   tyrosine to tryptophan or phenylalanine; valine to isoleucine
or leucine.

Additionally, other variants and fragments of IL-1δ or
IL-1ε can be used in the present invention. Variants include
analogs, homologues, derivatives, muteins, and mimetics of

IL-1δ or IL-1ε that retain the ability to block binding between
IL-1 family members and a target receptor. Fragments of the
IL-1δ or IL-1ε refer to portions of the amino acid sequence of
IL-1δ or IL-1ε as defined in SEQ ID NO: 2 or 4 that also retain
5   this ability. The variants and fragments can be generated
directly from IL-1δ or IL-1ε itself by chemical modification,
by proteolytic enzyme digestion, or by combinations thereof.
Additionally, genetic engineering techniques, as well as
methods of synthesizing polypeptides directly from amino acid
10   residues, can be employed.

        Non-peptide compounds that mimic the binding and function
of IL-1δ or IL-1ε ("mimetics") can be produced by the approach
outlined in Saragovi, et al. (1991) <u>Science</u> 253:792-95.
Mimetics are molecules which mimic elements of protein
15   secondary structure. See, e.g., Johnson et al., "Peptide Turn
Mimetics," in Pezzuto, et al. (eds. 1993) <u>Biotechnology and
Pharmacy</u>, Chapman and Hall, New York. The underlying
rationale behind the use of peptide mimetics is that the
peptide backbone of proteins exists chiefly to orient amino
20   acid side chains in such a way as to facilitate molecular
interactions. For the purposes of the present invention,
appropriate mimetics can be considered to be the equivalent of
IL-1δ or IL-1ε itself.

        Variants and fragments also can be created by recombinant
25   techniques employing genomic or cDNA cloning methods. Site-
specific and region-directed mutagenesis techniques can be
employed. See, e.g., vol. 1, ch. 8 in Ausubel, et al. (eds.
1989 and periodic updates) <u>Current Protocols in Molecular
Biology</u> Wiley and Sons; and Oxender and Fox (eds.) <u>Protein
30   Engineering</u> Liss, Inc. In addition, linker-scanning and PCR-
mediated techniques can be employed for mutagenesis. See,
e.g., Erlich (ed. 1989) <u>PCR Technology</u> Stockton Press.
Protein sequencing, structure and modeling approaches for use
with any of the above techniques are disclosed, e.g., in

Oxender and Fox (eds.) Protein Engineering Liss, Inc; and
Ausubel, et al. (eds. 1989 and periodic updates) Current
Protocols in Molecular Biology Wiley and Sons.

    This invention also contemplates the use of derivatives
5   of IL-1δ other than variations in amino acid sequence or
glycosylation. Such derivatives may involve covalent or
aggregative association with chemical moieties. These
derivatives generally fall into three classes: (1) salts, (2)
side chain and terminal residue covalent modifications, and
10 (3) adsorption complexes, e.g., with cell membranes. Such
covalent or aggregative derivatives are useful as immunogens,
as reagents in immunoassays, or in purification methods such
as for affinity purification of a receptor or other binding
molecule, e.g., an antibody. For example, an IL-1δ ligand can
15 be immobilized by covalent bonding to a solid support such as
cyanogen bromide-activated SEPHAROSE, by methods which are
well known in the art, or adsorbed onto polyolefin surfaces,
with or without glutaraldehyde cross-linking, for use in the
assay or purification of IL-1δ receptor, antibodies, or other
20 similar molecules. The IL-1δ can also be labeled with a
detectable group, e.g., radio-iodinated by the chloramine T
procedure, covalently bound to rare earth chelates, or
conjugated to another fluorescent moiety for use in diagnostic
assays.

25   An IL-1δ of this invention can be used as an immunogen
for the production of antisera or antibodies specific, e.g.,
capable of distinguishing between other IL-1 family members
and an IL-1δ, for the interleukin or any fragments thereof.
The purified interleukin can be used to screen monoclonal
30 antibodies or antigen-binding fragments prepared by
immunization with various forms of impure preparations
containing the protein. In particular, the term "antibodies"
also encompasses antigen binding fragments of natural
antibodies. The purified interleukin can also be used as a

reagent to detect any antibodies generated in response to the
presence of elevated levels of expression, or immunological
disorders which lead to antibody production to the endogenous
cytokine. Additionally, IL-1δ fragments may also serve as
5 immunogens to produce the antibodies of the present invention,
as described immediately below. For example, this invention
contemplates antibodies having binding affinity to or being
raised against the amino acid sequence shown in SEQ ID NO:2,
fragments thereof, or homologous peptides. In particular,
10 this invention contemplates antibodies having binding affinity
to, or having been raised against, specific fragments which
are predicted to be, or actually are, exposed at the exterior
protein surface of the native cytokine.

The blocking of physiological response to these
15 interleukins may result from the inhibition of binding of the
ligand to the receptor, likely through competitive inhibition.
Thus, in vitro assays of the present invention will often use
antibodies or ligand binding segments of these antibodies, or
fragments attached to solid phase substrates. These assays
20 will also allow for the diagnostic determination of the
effects of either binding region mutations and modifications,
or ligand mutations and modifications, e.g., ligand analogs.

This invention also contemplates the use of competitive
drug screening assays, e.g., where neutralizing antibodies to
25 the interleukin or fragments compete with a test compound for
binding to a receptor or antibody. In this manner, the
neutralizing antibodies or fragments can be used to detect the
presence of any polypeptide which shares one or more binding
sites to a receptor and can also be used to occupy binding
30 sites on a receptor that might otherwise bind an interleukin.

V. Making Nucleic Acids and Protein
DNA which encodes the protein or fragments thereof can be
obtained by chemical synthesis, screening cDNA libraries, or

by screening genomic libraries prepared from a wide variety of
cell lines or tissue samples. Natural sequences can be
isolated using standard methods and the sequences provided
herein, e.g., in SEQ ID NO:1. Other species counterparts,
5   e.g., primate, can be identified by hybridization techniques,
or by various PCR techniques, combined with or by searching in
sequence databases.

     This DNA can be expressed in a wide variety of host cells
for the synthesis of a full-length interleukin or fragments
10   which can in turn, e.g., be used to generate polyclonal or
monoclonal antibodies; for binding studies; for construction
and expression of modified agonist/antagonist molecules; and
for structure/function studies. Each variant or its fragments
can be expressed in host cells that are transformed or
15   transfected with appropriate expression vectors. These
molecules can be substantially free of protein or cellular
contaminants, other than those derived from the recombinant
host, and therefore are particularly useful in pharmaceutical
compositions when combined with a pharmaceutically acceptable
20   carrier and/or dilutent. The protein, or portions thereof,
may be expressed as fusions with other proteins.

     Expression vectors are typically self-replicating DNA or
RNA constructs containing the desired receptor gene or its
fragments, usually operably linked to suitable genetic control
25   elements that are recognized in a suitable host cell. These
control elements are capable of effecting expression within a
suitable host. The specific type of control elements
necessary to effect expression will depend upon the eventual
host cell used. Generally, the genetic control elements can
30   include a prokaryotic promoter system or a eukaryotic promoter
expression control system, and typically include a
transcriptional promoter, an optional operator to control the
onset of transcription, transcription enhancers to elevate the
level of mRNA expression, a sequence that encodes a suitable

ribosome binding site, and sequences that terminate
transcription and translation. Expression vectors also
usually contain an origin of replication that allows the
vector to replicate independently of the host cell.

5   The vectors of this invention include those which contain
DNA which encodes a protein, as described, or a fragment
thereof encoding a functionally, e.g., biologically active,
equivalent polypeptide. The DNA can be under the control of a
viral promoter and can encode a selection marker. This

10   invention further contemplates use of such expression vectors
which are capable of expressing eukaryotic cDNA coding for
such a protein in a prokaryotic or eukaryotic host, where the
vector is compatible with the host and where the eukaryotic
cDNA coding for the receptor is inserted into the vector such

15   that growth of the host containing the vector expresses the
cDNA in question. Usually, expression vectors are designed
for stable replication in their host cells or for
amplification to greatly increase the total number of copies
of the desirable gene per cell. It is not always necessary to

20   require that an expression vector replicate in a host cell,
e.g., it is possible to effect transient expression of the
interleukin protein or its fragments in various hosts using
vectors that do not contain a replication origin that is
recognized by the host cell. It is also possible to use

25   vectors that cause integration of the protein encoding portion
or its fragments into the host DNA by recombination, e.g.,
downstream of heterologous promoters.

        Vectors, as used herein, comprise plasmids, viruses,
bacteriophage, integratable DNA fragments, and other vehicles

30   which enable the integration of DNA fragments into the genome
of the host. Expression vectors are specialized vectors which
contain genetic control elements that effect expression of
operably linked genes. Plasmids are the most commonly used
form of vector but other forms of vectors which serve an

equivalent function and which are, or become, known in the art
are suitable for use herein. See, e.g., Pouwels, et al. (1985
and Supplements) <u>Cloning Vectors: A Laboratory Manual</u>,
Elsevier, N.Y., and Rodriquez, et al. (eds) <u>Vectors: A Survey</u>
5   <u>of Molecular Cloning Vectors and Their Uses</u>, Buttersworth,
Boston, 1988, which are incorporated herein by reference.

Transformed cells are cells, preferably mammalian, that
have been transformed or transfected with receptor vectors
constructed using recombinant DNA techniques. Transformed
10   host cells usually express the desired protein or its
fragments, but for purposes of cloning, amplifying, and
manipulating its DNA, do not need to express the subject
protein. This invention further contemplates culturing
transformed cells in a nutrient medium, thus permitting the
15   interleukin to accumulate in the culture. The protein can be
recovered, either from the culture or from the culture medium.

For purposes of this invention, nucleic acid sequences
are operably linked when they are functionally related to each
other. For example, DNA for a pre-sequence or secretory
20   leader is operably linked to a polypeptide if it is expressed
as a pre-protein or participates in directing the polypeptide
to the cell membrane or in secretion of the polypeptide. A
promoter is operably linked to a coding sequence if it
controls the transcription of the polypeptide; a ribosome
25   binding site is operably linked to a coding sequence if it is
positioned to permit translation. Usually, operably linked
means contiguous and in reading frame, however, certain
genetic elements such as repressor genes are not contiguously
linked but still bind to operator sequences that in turn
30   control expression.

Suitable host cells include prokaryotes, lower
eukaryotes, and higher eukaryotes. Prokaryotes include both
gram negative and gram positive organisms, e.g., <u>E. coli</u> and
<u>B. subtilis</u>. Lower eukaryotes include yeasts, e.g., <u>S.</u>

cerevisiae and Pichia, and species of the genus Dictyostelium.
Higher eukaryotes include established tissue culture cell
lines from animal cells, both of non-mammalian origin, e.g.,
insect cells, and birds, and of mammalian origin, e.g., human,
5   primates, and rodents.

Prokaryotic host-vector systems include a wide variety of
vectors for many different species. As used herein, E. coli
and its vectors will be used generically to include equivalent
vectors used in other prokaryotes. A common vector for
10   amplifying DNA is pBR322 or many of its derivatives. Vectors
that can be used to express the receptor or its fragments
include, but are not limited to, such vectors as those
containing the lac promoter (pUC-series); trp promoter
(pBR322-trp); lpp promoter (the pIN-series); lambda-pP or pR
15   promoters (pOTS); or hybrid promoters such as ptac (pDR540).
See Brosius, et al. (1988) "Expression Vectors Employing
Lambda-, trp-, lac-, and lpp-derived Promoters", in Rodriguez
and Denhardt (eds.) Vectors: A Survey of Molecular Cloning
Vectors and Their Uses, Buttersworth, Boston, Chapter 10, pp.
20   205-236, which is incorporated herein by reference.

Lower eukaryotes, e.g., yeasts and Dictyostelium, may be
transformed with IL-1δ sequence containing vectors. For
purposes of this invention, the most common lower eukaryotic
host is the baker's yeast, Saccharomyces cerevisiae. It will
25   be used to generically represent lower eukaryotes although a
number of other strains and species are also available. Yeast
vectors typically consist of a replication origin (unless of
the integrating type), a selection gene, a promoter, DNA
encoding the receptor or its fragments, and sequences for
30   translation termination, polyadenylation, and transcription
termination. Suitable expression vectors for yeast include
such constitutive promoters as 3-phosphoglycerate kinase and
various other glycolytic enzyme gene promoters or such
inducible promoters as the alcohol dehydrogenase 2 promoter or

metallothionine promoter. Suitable vectors include
derivatives of the following types: self-replicating low copy
number (such as the YRp-series), self-replicating high copy
number (such as the YEp-series); integrating types (such as
5   the YIp-series), or mini-chromosomes (such as the YCp-series).

Higher eukaryotic tissue culture cells are normally the
preferred host cells for expression of the functionally active
interleukin protein. In principle, any higher eukaryotic
tissue culture cell line is workable, e.g., insect baculovirus
10   expression systems, whether from an invertebrate or vertebrate
source. However, mammalian cells are preferred.
Transformation or transfection and propagation of such cells
has become a routine procedure. Examples of useful cell lines
include HeLa cells, Chinese hamster ovary (CHO) cell lines,
15   baby rat kidney (BRK) cell lines, insect cell lines, bird cell
lines, and monkey (COS) cell lines. Expression vectors for
such cell lines usually include an origin of replication, a
promoter, a translation initiation site, RNA splice sites (if
genomic DNA is used), a polyadenylation site, and a
20   transcription termination site. These vectors also usually
contain a selection gene or amplification gene. Suitable
expression vectors may be plasmids, viruses, or retroviruses
carrying promoters derived, e.g., from such sources as from
adenovirus, SV40, parvoviruses, vaccinia virus, or
25   cytomegalovirus. Representative examples of suitable
expression vectors include pCDNA1; pCD, see Okayama, et al.
(1985) Mol. Cell Biol. 5:1136-1142; pMC1neo PolyA, see Thomas,
et al. (1987) Cell 51:503-512; and a baculovirus vector such
as pAC 373 or pAC 610.
30   For secreted proteins, an open reading frame usually
encodes a polypeptide that consists of a mature or secreted
product covalently linked at its N-terminus to a signal
peptide. The signal peptide is typically cleaved prior to
secretion of the mature, or active, polypeptide. The cleavage

site can be predicted with a high degree of accuracy from
empirical rules, e.g., von-Heijne (1986) <u>Nucleic Acids
Research</u> 14:4683-4690, and the precise amino acid composition
of the signal peptide does not appear to be critical to its

5   function. See, e.g., Randall, et al. (1989) <u>Science</u> 243:1156-
1159; and Kaiser et al. (1987) <u>Science</u> 235:312-317.

     It will often be desired to express these polypeptides in
a system which provides a specific or defined glycosylation
pattern. In this case, the usual pattern will be that

10   provided naturally by the expression system. However, the
pattern will be modifiable by exposing the polypeptide, e.g.,
an unglycosylated form, to appropriate glycosylating proteins
introduced into a heterologous expression system. For
example, the interleukin gene may be co-transformed with one

15   or more genes encoding mammalian or other glycosylating
enzymes. Using this approach, certain mammalian glycosylation
patterns will be achievable in prokaryote or other cells.

     The source of IL-1δ or IL-1ϵ can be a eukaryotic or
prokaryotic host expressing recombinant IL-1δ or IL-1ϵ DNA,

20   such as is described above. The source can also be a cell
line such as mouse Swiss 3T3 fibroblasts, but other mammalian
cell lines are also contemplated by this invention, with the
preferred cell line being from the human species.

     Now that the entire sequence is known, the human IL-1δ,

25   fragments, or derivatives thereof can be prepared by
conventional processes for synthesizing peptides. These
include processes such as are described in Stewart and Young
(1984) <u>Solid Phase Peptide Synthesis</u>, Pierce Chemical Co.,
Rockford, IL; Bodanszky and Bodanszky (1984) <u>The Practice of

30   Peptide Synthesis</u>, Springer-Verlag, New York; and Bodanszky
(1984) <u>The Principles of Peptide Synthesis</u>, Springer-Verlag,
New York; all of each which are incorporated herein by
reference. For example, an azide process, an acid chloride
process, an acid anhydride process, a mixed anhydride process,

an active ester process (e.g., p-nitrophenyl ester,
N-hydroxysuccinimide ester, or cyanomethyl ester), a
carbodiimidazole process, an oxidative-reductive process, or a
dicyclohexylcarbodiimide (DCCD)/additive process can be used.
5   Solid phase and solution phase syntheses are both applicable
to the foregoing processes. Similar techniques can be used
with the IL-1ε or receptor sequences.

        The IL-1δ protein, fragments, or derivatives are suitably
prepared in accordance with the above processes as typically
10 employed in peptide synthesis, generally either by a so-called
stepwise process which comprises condensing an amino acid to
the terminal amino acid, one by one in sequence, or by
coupling peptide fragments to the terminal amino acid. Amino
groups that are not being used in the coupling reaction
15 typically must be protected to prevent coupling at an
incorrect location.

        If a solid phase synthesis is adopted, the C-terminal
amino acid is bound to an insoluble carrier or support through
its carboxyl group. The insoluble carrier is not particularly
20 limited as long as it has a binding capability to a reactive
carboxyl group. Examples of such insoluble carriers include
halomethyl resins, such as chloromethyl resin or bromomethyl
resin, hydroxymethyl resins, phenol resins,
tert-alkyloxycarbonylhydrazidated resins, and the like.

25   An amino group-protected amino acid is bound in sequence
through condensation of its activated carboxyl group and the
reactive amino group of the previously formed peptide or
chain, to synthesize the peptide step by step. After
synthesizing the complete sequence, the peptide is split off
30 from the insoluble carrier to produce the peptide. This
solid-phase approach is generally described by Merrifield, et
al. (1963) in J. Am. Chem. Soc. 85:2149-2156, which is
incorporated herein by reference.

The prepared protein and fragments thereof can be
isolated and purified from the reaction mixture by means of
peptide separation, e.g., by extraction, precipitation,
electrophoresis, various forms of chromatography, and the
5   like. The interleukin of this invention can be obtained in
varying degrees of purity depending upon its desired use.
Purification can be accomplished by use of the protein
purification techniques disclosed herein, see below, or by the
use of the antibodies herein described in methods of
10   immunoabsorbant affinity chromatography. This immunoabsorbant
affinity chromatography is carried out by first linking the
antibodies to a solid support and then contacting the linked
antibodies with solubilized lysates of appropriate cells,
lysates of other cells expressing the interleukin, or lysates
15   or supernatants of cells producing the protein as a result of
DNA techniques, see below.

Generally, the purified protein will be at least about
40% pure, ordinarily at least about 50% pure, usually at least
about 60% pure, typically at least about 70% pure, more
20   typically at least about 80% pure, preferable at least about
90% pure and more preferably at least about 95% pure, and in
particular embodiments, 97%-99% or more. Purity will usually
be on a weight basis, but can also be on a molar basis.
Different assays will be applied as appropriate.

25

VI.   Antibodies

The term "antibody" or "antibody molecule" as used in
this invention includes intact molecules as well as fragments
thereof, such as Fab, F(ab')$_2$, and Fv which are capable of
30   selectively binding the epitopic determinant. These antibody
fragments retain some ability to selectively bind with its
antigen or receptor and are defined as follows: (1) Fab, the
fragment which contains a monovalent antigen-binding fragment
of an antibody molecule can be produced by digestion of whole

antibody with the enzyme papain to yield an intact light chain
and a portion of one heavy chain; (2) Fab', the fragment of an
antibody molecule can be obtained by treating whole antibody
with pepsin, followed by reduction, to yield an intact light
5   chain and a portion of the heavy chain; two Fab' fragments are
obtained per antibody molecule; (3) (Fab')$_2$, the fragment of
the antibody that can be obtained by treating whole antibody
with the enzyme pepsin without subsequent reduction; F(ab')$_2$
is a dimer of two Fab' fragments held together by two
10   disulfide bonds; (4) Fv, defined as a genetically engineered
fragment containing the variable region of the light chain and
the variable region of the heavy chain expressed as two
chains; and (5) Single chain antibody ("SCA"), defined as a
genetically engineered molecule containing the variable region
15   of the light chain, the variable region of the heavy chain,
linked by a suitable polypeptide linker as a genetically fused
single chain molecule.

     Methods of making these fragments are known in the art.
See, e.g., Harlow and Lane (current edition) Antibodies: A
20   Laboratory Manual, Cold Spring Harbor Laboratory, New York.
Therefore, the phrase "antibody molecule" in its various forms
as used herein contemplates both an intact antibody
(immunoglobulin) molecule and an immunologically active
portion of an antibody (immunoglobulin) molecule. Recombinant
25   methods may be applied to make these fragments.

     The term "monoclonal antibody" refers to a population of
one species of antibody molecule of antigen-specificity. A
monoclonal antibody contains only one species of antibody
combining site capable of immunoreacting with a particular
30   antigen and thus typically displays a single binding affinity
for that antigen. A monoclonal antibody may therefore contain
a bispecific antibody molecule having two antibody combining
sites, each immunospecific for a different antigen. In one

embodiment, the first antibody molecule is affixed to a solid
support.

     As used in this invention, the term "epitope" means an
antigenic determinant on an antigen to which the paratope of
5   an antibody binds. Epitopic determinants usually consist of
chemically active surface groupings such as amino acids or
sugar side chains and usually have specific three dimensional
structural characteristics, as well as specific charge
characteristics.

10   The word "complex" as used herein refers to the product
of a specific binding agent-target reaction. An exemplary
complex is an immunoreaction product formed by an antibody-
antigen reaction.

     The term "antigen" refers to a polypeptide or protein
15   that is able to specifically bind to (immunoreact with) an
antibody and form an immunoreaction product (immunocomplex).
The site on the antigen with which the antibody binds is
referred to as an antigenic determinant or epitope, and the
binding should be detectable, e.g., 2X, 5X or more above
20   background.

     A method of the invention for detection of antibodies
that bind to novel epitopes in a sample is performed in vitro,
e.g., in immunoassays in which the antibodies can be
identified in liquid phase or bound to a solid phase carrier.
25   In various embodiments, the method is performed with a capture
antibody bound to a solid support, and/or the capture antibody
is a monoclonal antibody molecule.

     Examples of types of immunoassays which can be utilized
to detect novel antibodies in a sample, include competitive
30   and non-competitive immunoassays in either a direct or
indirect format. Examples of such immunoassays are the
radioimmunoassay (RIA) and the sandwich (immunometric) assay.
Detection of the antibodies can be done utilizing immunoassays
which are run in either the forward, reverse, or simultaneous

modes, including competition immunoassays and
immunohistochemical assays on physiological samples.
Preferably, the method of the invention utilizes a forward
immunoassay. Those of skill in the art will know, or can
5   readily discern, other immunoassay formats without undue
experimentation.

Solid phase-bound antibody molecules are bound by
adsorption from an aqueous medium, although other modes of
affixation, such as covalent coupling or other well known
10   means of affixation to the solid matrix can be used.
Preferably, the first antibody molecule is bound to a support
before forming an immunocomplex with antigen, however, the
immunocomplex can be formed prior to binding the complex to
the solid support.

15   Non-specific protein binding sites on the surface of the
solid phase support are preferably blocked. After adsorption
of solid phase-bound antibodies, an aqueous solution of a
protein free from interference with the assay such as bovine,
horse, or other serum albumin that is also free from
20   contamination with the antigen is admixed with the solid phase
to adsorb the admixed protein onto the surface of the
antibody-containing solid support at protein binding sites on
the surface that are not occupied by the antibody molecule.

A typical aqueous protein solution contains about 2-10
25   weight percent bovine serum albumin in PBS at a pH of about 7-
8. The aqueous protein solution-solid support mixture is
typically maintained for a time period of at least one hour at
a temperature of about 4˚-37˚ C and the resulting solid phase
is thereafter rinsed free of unbound protein.

30   The first preselected antibody can be bound to many
different carriers and used to detect novel epitope binding
antibodies in a sample. Examples of well-known carriers
include glass, polystyrene, polypropylene, polyethylene,
dextran, nylon, amyloses, natural and modified celluloses,

polyacrylamides, agaroses, and magnetite. The nature of the
carrier can be either soluble or insoluble for purposes of the
invention. Those skilled in the art will know of other
suitable carriers for binding antibodies, or will be able to
5   ascertain such, using routine experimentation.

In addition, if desirable, an antibody for detection in
these immunoassays can be detectably labeled in various ways.
There are many different labels and methods of labeling known.
Examples of the types of labels which can be used in the
10   present invention include, e.g., enzymes, radioisotopes,
fluorescent compounds, colloidal metals, chemiluminescent
compounds, and bio-luminescent compounds. Many other suitable
labels exist for binding to the monoclonal antibodies of the
invention

15   Antibodies which bind to IL-1δ or IL-1ε polypeptides of
the invention can be prepared using an intact polypeptide or
fragments containing peptides of interest as the immunizing
antigen. The polypeptide or a peptide used to immunize an
animal can be derived from translated cDNA or chemical
20   synthesis which can be conjugated to a carrier protein, if
desired. Such commonly used carriers which are chemically
coupled to the peptide include keyhole limpet hemocyanin
(KLH), thyroglobulin, bovine serum albumin (BSA), and tetanus
toxoid. The coupled peptide is then used to immunize the host
25   animal, e.g., a mouse, a rat, or a rabbit.

If desired, polyclonal or monoclonal antibodies can be
further purified, e.g., by binding to and elution from a
matrix to which is bound the antigen to which the antibodies
were raised. Many techniques are available for purification
30   and/or concentration of polyclonal antibodies, as well as
monoclonal antibodies. See, e.g., Coligan, et al. (current
ed.) Unit 9, Current Protocols in Immunology, Wiley
Interscience.

It is also possible to use the anti-idiotype antibody technology to produce monoclonal antibodies which mimic an epitope. For example, an anti-idiotypic monoclonal antibody made to a first monoclonal antibody will have a binding domain
5   in the hypervariable region which is the "image" of the epitope bound by the first monoclonal antibody.

The preparation of polyclonal antibodies is well-known to those skilled in the art. See, e.g., Green, et al. "Production of Polyclonal Antisera" pages 1-5 in Manson (ed.)
10   Immunochemical Protocols Humana Press; Production of Polyclonal Antisera in Rabbits, Rats, Mice and Hamsters section 2.4.1 in Coligan, et al. Current Protocols in Immunology.

The preparation of monoclonal antibodies likewise is
15   conventional. See, e.g., Kohler and Milstein (1975) Nature 256:495-497; Coligan, et al. Current Protocols sections 2.5.1-2.6.7; and Harlow and Lane (1989). Briefly, monoclonal antibodies can be obtained by injecting mice with a composition comprising an antigen, verifying the presence of
20   antibody production by removing a serum sample, removing the spleen to obtain B lymphocytes, fusing the B lymphocytes with myeloma cells to produce hybridomas, cloning the hybridomas, selecting positive clones that produce antibodies to the antigen, and isolating the antibodies from the hybridoma
25   cultures. Monoclonal antibodies can be isolated and purified from hybridoma cultures by a variety of well-established techniques. Such isolation techniques include affinity chromatography with Protein-A Sepharose, size-exclusion chromatography, and ion-exchange chromatography. See, e.g.,
30   Coligan, et al. Current Protocols sections 2.7.1-2.7.12 and 2.9.1-2.9.3; Barnes, et al. in Methods in Molecular Biology, Humana Press.

Therapeutic applications are conceivable for the antibodies of the present invention. For example, antibodies

of the present invention may also be derived from subhuman
primate antibody. General techniques for raising
therapeutically useful antibodies in baboons may be found,
e.g., in Goldenberg, et al. (1991) WO 91/11465; and Losman, et
5   al. (1990) Int. J. Cancer 46:310-314.

Alternatively, a therapeutically useful anti-IL-1δ or
anti-IL-1ε antibody may be derived from a "humanized"
monoclonal antibody. Humanized monoclonal antibodies are
produced by transferring mouse complementary determining
10 regions from heavy and light variable chains of the mouse
immunoglobulin into a human variable domain, and then
substituting human residues in the framework regions of the
murine counterparts. The use of antibody components derived
from humanized monoclonal antibodies obviates potential
15 problems associated with the immunogenicity of murine constant
regions. General techniques for cloning murine immunoglobulin
variable domains are described, e.g., by Orlandi, et al.
(1989) Proc. Nat'l Acad. Sci. USA 86:3833-3837. Techniques
for producing humanized monoclonal antibodies are described,
20 e.g., by Jones, et al. (1986) Nature 321:522-525; Riechmann,
et al. (1988) Nature 332:323-327; Verhoeyen, et al. (1988)
Science 239:1534-1536; Carter, et al. (1992) Proc. Nat'l Acad.
Sci. USA 89:4285-4289; Sandhu (1992) Crit. Rev. Biotech.
12:437-462; and Singer, et al. (1993) J. Immunol. 150:2844-
25 2857.

Antibodies of the invention also may be derived from
human antibody fragments isolated from a combinatorial
immunoglobulin library. See, e.g., Barbas, et al. (1991)
Methods: A Companion to Methods in Enzymology, vol. 2, page
30 119; and Winter, et al. (1994) Ann. Rev. Immunol. 12:433-465.
Cloning and expression vectors that are useful for producing a
human immunoglobulin phage library can be obtained, e.g., from
STRATAGENE Cloning Systems (La Jolla, CA).

In addition, antibodies of the present invention may be derived from a human monoclonal antibody. Such antibodies are obtained from transgenic mice that have been "engineered" to produce specific human antibodies in response to antigenic
5   challenge. In this technique, elements of the human heavy and light chain loci are introduced into strains of mice derived from embryonic stem cell lines that contain targeted disruptions of the endogenous heavy and light chain loci. The transgenic mice can synthesize human antibodies specific for
10   human antigens, and the mice can be used to produce human antibody-secreting hybridomas. Methods for obtaining human antibodies from transgenic mice are described by Green, et al. (1994) Nature Genet. 7:13-21; Lonberg, et al. (1994) Nature 368:856-859; and Taylor, et al. (1994) Int. Immunol. 6:579-
15   591.

Antibody fragments of the present invention can be prepared by proteolytic hydrolysis of the antibody or by expression in E. coli of DNA encoding the fragment. Antibody fragments can be obtained by pepsin or papain digestion of
20   whole antibodies by conventional methods. For example, antibody fragments can be produced by enzymatic cleavage of antibodies with pepsin to provide a fragment denoted $F(ab')_2$. This fragment can be further cleaved using a thiol reducing agent, and optionally a blocking group for the sulfhydryl
25   groups resulting from cleavage of disulfide linkages, to produce Fab' monovalent fragments. Alternatively, an enzymatic cleavage using papain produces two monovalent Fab fragments and an Fc fragment directly. These methods are described, e.g., by Goldenberg, U.S. patents No. 4,036,945 and
30   No. 4,331,647, and references contained therein. These patents are hereby incorporated in their entireties by reference including all figures, drawings, and illustrations. See also Nisonhoff, et al. (1960) Arch. Biochem. Biophys. 89:230-et seq.; Porter (1959) Biochem. J. 73:119-et seq.;

Edelman, et al. (1967) Methods in Enzymology, vol. 1, Academic Press; and Coligan, et al. Current Protocols, at sections 2.8.1-2.8.10 and 2.10.1-2.10.4.

Other methods of cleaving antibodies, such as separation
5 of heavy chains to form monovalent light-heavy chain fragments, further cleavage of fragments, or other enzymatic, chemical, or genetic techniques may also be used, so long as the fragments bind to the antigen that is recognized by the intact antibody.

10   For example, Fv fragments comprise an association of V_H and V_L chains. This association may be noncovalent, as described in Inbar, et al. (1972) Proc. Nat'l Acad. Sci. USA 69:2659-et seq. Alternatively, the variable chains can be linked by an intermolecular disulfide bond or cross-linked by
15 chemicals such as glutaraldehyde. See, e.g., Sandhu (1992) Crit. Rev. Biotech. 12:437-462. Preferably, the Fv fragments comprise V_H and V_L chains connected by a peptide linker. These single-chain antigen binding proteins (sFv) are prepared by constructing a structural gene comprising DNA sequences
20 encoding the V_H and V_L domains connected by an oligonucleotide. The structural gene is inserted into an expression vector, which is subsequently introduced into a host cell such as E. coli. The recombinant host cells synthesize a single polypeptide chain with a linker peptide
25 bridging the two V domains. Methods for producing sFvs are described, e.g., by Whitlow, et al. (1991) Methods: A Companion to Methods in Enzymology, vol. 2, page 97; Bird, et al. (1988) Science 242:423-426; Ladner, et al., U.S. patent No. 4,946,778; Pack, et al. (1993) Bio/Technology 11:1271-77;
30 and Sandhu (1992) Crit. Rev. Biotech. 12:437-462.

Another form of an antibody fragment is a peptide coding for a single complementarity-determining region (CDR). CDR peptides ("minimal recognition units") can be obtained by

constructing genes encoding the CDR of an antibody of
interest. Such genes are prepared, e.g., by using the
polymerase chain reaction to synthesize the variable region
from RNA of antibody-producing cells. See, e.g., Larrick, et
5 al. (1991) <u>Methods: A Companion to Methods in Enzymology</u>, vol.
2, page 106.

Antibodies can be raised to the various mammalian, e.g.,
IL-1δ and/or IL-1ε proteins and fragments thereof, both in
naturally occurring native forms and in their recombinant
10 forms, the difference being that antibodies to the active
ligand are more likely to recognize epitopes which are only
present in the native conformations. Denatured antigen
detection can also be useful in, e.g., Western blot analysis.
Anti-idiotypic antibodies are also contemplated, which would
15 be useful as agonists or antagonists of a natural receptor or
an antibody.

A number of immunogens may be used to produce antibodies
specifically reactive with thymokine proteins. Recombinant
protein is the preferred immunogen for the production of
20 monoclonal or polyclonal antibodies. Naturally occurring
protein may also be used either in pure or impure form.
Synthetic peptides made using the human or mouse lymphotactin
protein sequences described herein may also used as an
immunogen for the production of antibodies to thymokines.
25 Recombinant protein can be expressed in eukaryotic or
prokaryotic cells as described herein, and purified as
described. The product is then injected into an animal
capable of producing antibodies. Either monoclonal or
polyclonal antibodies may be generated for subsequent use in
30 immunoassays to measure the protein.

Methods of producing polyclonal antibodies are known to
those of skill in the art. In brief, an immunogen, preferably
a purified protein, is mixed with an adjuvant and animals are
immunized with the mixture. The animal's immune response to

the immunogen preparation is monitored by taking test bleeds
and determining the titer of reactivity to the antigen of
interest. When appropriately high titers of antibody to the
immunogen are obtained, blood is collected from the animal and
5   antisera are prepared. Further fractionation of the antisera
to enrich for antibodies reactive to the protein can be done
if desired. See Harlow and Lane.

Monoclonal antibodies may be obtained by various
techniques familiar to those skilled in the art. Briefly,
10 spleen cells from an animal immunized with a desired antigen
are immortalized, commonly by fusion with a myeloma cell.
Alternative methods of immortalization include transformation
with Epstein Barr Virus, oncogenes, or retroviruses, or other
methods well known in the art. Colonies arising from single
15 immortalized cells are screened for production of antibodies
of the desired specificity and affinity for the antigen, and
yield of the monoclonal antibodies produced by such cells may
be enhanced by various techniques, including injection into
the peritoneal cavity of a vertebrate host. Alternatively,
20 one may isolate DNA sequences which encode a monoclonal
antibody or a binding fragment thereof by screening a DNA
library from human B cells according to the general protocol
outlined by Huse, et al. (1989) Science 246:1275-1281.

Antibodies, including binding fragments and single chain
25 versions, against predetermined fragments of the protein can
be raised by immunization of animals with conjugates of the
fragments with immunogenic proteins. Monoclonal antibodies
are prepared from cells secreting the desired antibody. These
antibodies can be screened for binding to normal or defective
30 protein, or screened for agonistic or antagonistic activity.
These monoclonal antibodies will usually bind with at least a
K_D of about 1 mM, more usually at least about 300 µM,
typically at least about 100 µM, more typically at least about
30 µM, preferably at least about 10 µM, and more preferably at

least about 3 µM or better; including 1 µM, 300 nM, 100 nM, 30 nM, etc.

The antibodies, including antigen binding fragments, of this invention can have significant diagnostic or therapeutic

5   value. They can be potent antagonists that bind to the interleukin and inhibit binding to the receptor or inhibit the ability of IL-1δ or IL-1ε to elicit a biological response. They also can be useful as non-neutralizing antibodies and can be coupled to toxins or radionuclides to bind producing cells,

10   or cells localized to the source of the interleukin. Further, these antibodies can be conjugated to drugs or other therapeutic agents, either directly or indirectly by means of a linker.

The antibodies of this invention can also be useful in

15   diagnostic applications. As capture or non-neutralizing antibodies, they can bind to the interleukin without inhibiting receptor binding. As neutralizing antibodies, they can be useful in competitive binding assays. They will also be useful in detecting or quantifying IL-1δ. They may be used

20   as reagents for Western blot analysis, or for immunoprecipitation or immunopurification of the respective protein.

Protein fragments may be joined to other materials, particularly polypeptides, as fused or covalently joined

25   polypeptides to be used as immunogens. Primate IL-1δ and its fragments may be fused or covalently linked to a variety of immunogens, such as keyhole limpet hemocyanin, bovine serum albumin, tetanus toxoid, etc. See <u>Microbiology</u>, Hoeber Medical Division, Harper and Row, 1969; Landsteiner (1962)

30   <u>Specificity of Serological Reactions</u>, Dover Publications, New York; and Williams, et al. (1967) <u>Methods in Immunology and Immunochemistry</u>, Vol. 1, Academic Press, New York; each of which are incorporated herein by reference, for descriptions of methods of preparing polyclonal antisera. A typical method

involves hyperimmunization of an animal with an antigen. The
blood of the animal is then collected shortly after the
repeated immunizations and the gamma globulin is isolated.

In some instances, it is desirable to prepare monoclonal
5   antibodies from various mammalian hosts, such as mice,
rodents, primates, humans, etc. Description of techniques for
preparing such monoclonal antibodies may be found in, e.g.,
Stites, et al. (eds) Basic and Clinical Immunology (4th ed.),
Lange Medical Publications, Los Altos, CA, and references
10   cited therein; Harlow and Lane (1988) Antibodies: A
Laboratory Manual, CSH Press; Goding (1986) Monoclonal
Antibodies: Principles and Practice (2d ed) Academic Press,
New York; and particularly in Kohler and Milstein (1975) in
Nature 256:495-497, which discusses one method of generating
15   monoclonal antibodies. Each of these references is
incorporated herein by reference. Summarized briefly, this
method involves injecting an animal with an immunogen. The
animal is then sacrificed and cells taken from its spleen,
which are then fused with myeloma cells. The result is a
20   hybrid cell or "hybridoma" that is capable of reproducing in
vitro. The population of hybridomas is then screened to
isolate individual clones, each of which secrete a single
antibody species to the immunogen. In this manner, the
individual antibody species obtained are the products of
25   immortalized and cloned single B cells from the immune animal
generated in response to a specific site recognized on the
immunogenic substance.

Other suitable techniques involve in vitro exposure of
lymphocytes to the antigenic polypeptides or alternatively to
30   selection of libraries of antibodies in phage or similar
vectors. See, Huse, et al. (1989) "Generation of a Large
Combinatorial Library of the Immunoglobulin Repertoire in
Phage Lambda," Science 246:1275-1281; and Ward, et al. (1989)
Nature 341:544-546, each of which is hereby incorporated

herein by reference. The polypeptides and antibodies of the
present invention may be used with or without modification,
including chimeric or humanized antibodies. Frequently, the
polypeptides and antibodies will be labeled by joining, either
5   covalently or non-covalently, a substance which provides for a
detectable signal. A wide variety of labels and conjugation
techniques are known and are reported extensively in both the
scientific and patent literature. Suitable labels include
radionuclides, enzymes, substrates, cofactors, inhibitors,
10 fluorescent moieties, chemiluminescent moieties, magnetic
particles, and the like. Patents, teaching the use of such
labels include U.S. Patent Nos. 3,817,837; 3,850,752;
3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241.
Also, recombinant or chimeric immunoglobulins may be produced,
15 see Cabilly, U.S. Patent No. 4,816,567; or made in transgenic
mice, see Mendez, et al. (1997) Nature Genetics 15:146-156.
These references are incorporated herein by reference.

The antibodies of this invention can also be used for
affinity chromatography in isolating primate IL-1δ. Columns
20 can be prepared where the antibodies are linked to a solid
support, e.g., particles, such as agarose, SEPHADEX, or the
like, where a cell lysate may be passed through the column,
the column washed, followed by increasing concentrations of a
mild denaturant, whereby the purified protein will be
25 released. Conversely, protein may be used to purify antibody.

Antibodies may also be used to screen expression
libraries for particular expression products. Usually
antibodies used in such a procedure will be labeled with a
moiety allowing easy detection of presence of antigen by
30 antibody binding.

Antibodies raised against an IL-1δ or IL-1ε will also be
used to raise anti-idiotypic antibodies. These will be useful
in detecting or diagnosing various immunological conditions
related to expression of the protein or cells which express

receptors for the protein. They also will be useful as
agonists or antagonists of the interleukin, which may be
competitive inhibitors or substitutes for naturally occurring
ligands.

5

Binding Agent:IL-1δ/IL-1ε Protein Complex

       An IL-1δ or IL-1ε protein that specifically binds to or
that is specifically immunoreactive with an antibody, e.g., a
polyclonal antibody generated against a defined immunogen,
10   e.g., an immunogen consisting of an amino acid sequence of SEQ
ID NO: 2, is typically determined in an immunoassay. Included
within the present invention are those nucleic acid sequences
described herein, including functional variants, that encode
polypeptides that bind to polyclonal antibodies generated
15   against the prototypical primate IL-1δ, but not the prior
known rodent counterpart. The immunoassay typically uses a
polyclonal antiserum which was raised, e.g., to a protein of
SEQ ID NO: 2. This antiserum is selected to have low
crossreactivity against other IL-1 family members, preferably
20   from the same species, and to other rodent or similar
evolutionarily distant IL-1δ, so that any such crossreactivity
is removed by immunoabsorption prior to use in the
immunoassay.

       In order to produce antisera for use in an immunoassay,
25   the protein of SEQ ID NO: 2 is isolated as described herein.
For example, recombinant protein may be produced in a
mammalian cell line. An appropriate host, e.g., an inbred
strain of mice such as Balb/c, is immunized with the protein
of SEQ ID NO: 2 using a standard adjuvant, such as Freund's
30   adjuvant, and a standard mouse immunization protocol (see
Harlow and Lane). Alternatively, a synthetic peptide derived
from the sequences disclosed herein and conjugated to a
carrier protein can be used an immunogen. Polyclonal sera are
collected, perhaps immunodepleted, and titered against the

immunogen protein in an immunoassay, e.g., a solid phase
immunoassay with the immunogen immobilized on a solid support.
Polyclonal antisera with a titer of 10^4 or greater are
selected and tested for their cross reactivity against other

5   IL-1 family members, e.g., IL-1α, IL-1β, IL-1RA, IL-1γ, IL-1ε,
and rodent IL-1δ using a competitive binding immunoassay such
as the one described in Harlow and Lane, supra, at pages 570-
573.   Preferably at least two IL-1 family members are used in
this determination in conjunction with rodent IL-1δ.   These

10   IL-1 family members can be produced as recombinant proteins
and isolated using standard molecular biology and protein
chemistry techniques as described herein.

     Immunoassays in the competitive binding format can be
used for the crossreactivity determinations.   For example, the

15   protein of SEQ ID NO: 2 can be immobilized to a solid support.
Proteins added to the assay compete with the binding of the
antisera to the immobilized antigen.   The ability of the above
proteins to compete with the binding of the antisera to the
immobilized protein is compared to the protein of SEQ ID NO:

20   2.   The percent crossreactivity for the above proteins is
calculated, using standard calculations.   Those antisera with
less than 10% crossreactivity with each of the proteins listed
above are selected and pooled.   The cross-reacting antibodies
are then removed from the pooled antisera by immunoabsorbtion

25   with the above-listed proteins.

     The immunoabsorbed and pooled antisera are then used in a
competitive binding immunoassay as described above to compare
a second protein to the immunogen protein (e.g., the IL-1 like
protein of SEQ ID NO: 2).   In order to make this comparison,

30   the two proteins are each assayed at a wide range of
concentrations and the amount of each protein required to
inhibit 50% of the binding of the antisera to the immobilized
protein is determined.   If the amount of the second protein
required is less than twice the amount of the protein of SEQ

ID NO: 2 that is required, then the second protein is said to specifically bind to an antibody generated to the immunogen.

It is understood that these IL-1δ or IL-1ε proteins are members of a family of homologous proteins that comprise at

5   least 5 so far identified genes. For a particular gene product, such as the IL-1δ or IL-1ε protein, the term refers not only to the amino acid sequences disclosed herein, but also to other proteins that are allelic, non-allelic or species variants. It is also understood that the term "IL-1δ"

10 or "IL-1ε" includes nonnatural mutations introduced by deliberate mutation using conventional recombinant technology such as single site mutation, or by excising short sections of DNA encoding the respective proteins, or by substituting new amino acids, or adding new amino acids. Such minor

15 alterations must substantially maintain the immunoidentity of the original molecule and/or its biological activity. Thus, these alterations include proteins that are specifically immunoreactive with a designated naturally occurring IL-1 related protein, e.g., the IL-1δ protein shown in SEQ ID NO:

20 2. The biological properties of the altered proteins can be determined by expressing the protein in an appropriate cell line and measuring the appropriate effect upon lymphocytes. Particular protein modifications considered minor would include conservative substitution of amino acids with similar

25 chemical properties, as described above for the IL-1 family as a whole. By aligning a protein optimally with the protein of SEQ ID NO: 2 and by using the conventional immunoassays described herein to determine immunoidentity, one can determine the protein compositions of the invention.

30

VII.   Kits and quantitation

Both naturally occurring and recombinant forms of the IL-1 like molecules of this invention are particularly useful in kits and assay methods. For example, these methods would also

be applied to screening for binding activity, e.g., receptors
for these proteins. Several methods of automating assays have
been developed in recent years so as to permit screening of
tens of thousands of compounds per year. See, e.g., a BIOMEK
5   automated workstation, Beckman Instruments, Palo Alto,
California, and Fodor, et al. (1991) Science 251:767-773,
which is incorporated herein by reference. The latter
describes means for testing binding by a plurality of defined
polymers synthesized on a solid substrate. The development of
10 suitable assays to screen for a receptor or agonist/antagonist
homologous proteins can be greatly facilitated by the
availability of large amounts of purified, soluble IL-1δ or
IL-1ε in an active state such as is provided by this
invention.

15   Purified IL-1δ can be coated directly onto plates for use
in the aforementioned receptor screening techniques. However,
non-neutralizing antibodies to these proteins can be used as
capture antibodies to immobilize the respective interleukin on
the solid phase, useful, e.g., in diagnostic uses.

20   This invention also contemplates use of IL-1δ, fragments
thereof, peptides, and their fusion products in a variety of
diagnostic kits and methods for detecting the presence of the
protein or its receptor. Alternatively, or additionally,
antibodies against the molecules may be incorporated into the
25 kits and methods. Typically the kit will have a compartment
containing either a defined IL-1δ peptide or gene segment or a
reagent which recognizes one or the other. Typically,
recognition reagents, in the case of peptide, would be a
receptor or antibody, or in the case of a gene segment, would
30 usually be a hybridization probe.

        A preferred kit for determining the concentration of,
e.g., IL-1δ, a sample would typically comprise a labeled
compound, e.g., receptor or antibody, having known binding
affinity for IL-1δ, a source of IL-1δ (naturally occurring or

recombinant) as a positive control, and a means for separating
the bound from free labeled compound, e.g., a solid phase for
immobilizing the IL-1δ in the test sample. Compartments
containing reagents, and instructions, will normally be
5   provided.

Antibodies, including antigen binding fragments, specific
for mammalian IL-1δ or a peptide fragment, or receptor
fragments are useful in diagnostic applications to detect the
presence of elevated levels of IL-1δ and/or its fragments.
10   Diagnostic assays may be homogeneous (without a separation
step between free reagent and antibody-antigen complex) or
heterogeneous (with a separation step). Various commercial
assays exist, such as radioimmunoassay (RIA), enzyme-linked
immunosorbent assay (ELISA), enzyme immunoassay (EIA),
15   enzyme-multiplied immunoassay technique (EMIT),
substrate-labeled fluorescent immunoassay (SLFIA) and the
like. For example, unlabeled antibodies can be employed by
using a second antibody which is labeled and which recognizes
the antibody to IL-1δ or to a particular fragment thereof.
20   These assays have also been extensively discussed in the
literature. See, e.g., Harlow and Lane (1988) <u>Antibodies: A
Laboratory Manual</u>, CSH., and Coligan (ed. 1991 and periodic
supplements) <u>Current Protocols In Immunology</u> Greene/Wiley, New
York.

25   Anti-idiotypic antibodies may have similar use to serve
as agonists or antagonists of IL-1δ. These should be useful
as therapeutic reagents under appropriate circumstances.

Frequently, the reagents for diagnostic assays are
supplied in kits, so as to optimize the sensitivity of the
30   assay. For the subject invention, depending upon the nature
of the assay, the protocol, and the label, either labeled or
unlabeled antibody, or labeled receptor is provided. This is
usually in conjunction with other additives, such as buffers,
stabilizers, materials necessary for signal production such as

substrates for enzymes, and the like. Preferably, the kit
will also contain instructions for proper use and disposal of
the contents after use. Typically the kit has compartments
for each useful reagent, and will contain instructions for
5   proper use and disposal of reagents. Desirably, the reagents
are provided as a dry lyophilized powder, where the reagents
may be reconstituted in an aqueous medium having appropriate
concentrations for performing the assay.

     Any of the aforementioned constituents of the diagnostic
10   assays may be used without modification or may be modified in
a variety of ways. For example, labeling may be achieved by
covalently or non-covalently joining a moiety which directly
or indirectly provides a detectable signal. In any of these
assays, a test compound, IL-1δ, or antibodies thereto can be
15   labeled either directly or indirectly. Possibilities for
direct labeling include label groups: radiolabels such as
^{125}I, enzymes (U.S. Pat. No. 3,645,090) such as peroxidase and
alkaline phosphatase, and fluorescent labels (U.S. Pat. No.
3,940,475) capable of monitoring the change in fluorescence
20   intensity, wavelength shift, or fluorescence polarization.
Both patents are incorporated herein by reference.
Possibilities for indirect labeling include biotinylation of
one constituent followed by binding to avidin coupled to one
of the above label groups.

25   There are also numerous methods of separating the bound
from the free ligand, or alternatively the bound from the free
test compound. The IL-1δ can be immobilized on various
matrixes followed by washing. Suitable matrices include
plastic such as an ELISA plate, filters, and beads. Methods
30   of immobilizing the receptor to a matrix include, without
limitation, direct adhesion to plastic, use of a capture
antibody, chemical coupling, and biotin-avidin. The last step
in this approach involves the precipitation of
antibody/antigen complex by any of several methods including

those utilizing, e.g., an organic solvent such as polyethylene
glycol or a salt such as ammonium sulfate. Other suitable
separation techniques include, without limitation, the
fluorescein antibody magnetizable particle method described in
5   Rattle, et al. (1984) Clin. Chem. 30(9):1457-1461, and the
double antibody magnetic particle separation as described in
U.S. Pat. No. 4,659,678, each of which is incorporated herein
by reference.

    The methods for linking protein or fragments to various
10   labels have been extensively reported in the literature and do
not require detailed discussion here. Many of the techniques
involve the use of activated carboxyl groups either through
the use of carbodiimide or active esters to form peptide
bonds, the formation of thioethers by reaction of a mercapto
15   group with an activated halogen such as chloroacetyl, or an
activated olefin such as maleimide, for linkage, or the like.
Fusion proteins will also find use in these applications.

    Another diagnostic aspect of this invention involves use
of oligonucleotide or polynucleotide sequences taken from the
20   sequence of a primate IL-1δ. These sequences can be used as
probes for detecting levels of the IL-1δ in patients suspected
of having an immunological disorder, or to evaluate
polymorphic variation. The preparation of both RNA and DNA
nucleotide sequences, the labeling of the sequences, and the
25   preferred size of the sequences has received ample description
and discussion in the literature. Normally an oligonucleotide
probe should have at least about 14 nucleotides, usually at
least about 18 nucleotides, and the polynucleotide probes may
be up to several kilobases. Various labels may be employed,
30   most commonly radionuclides, particularly ^{32}P. However, other
techniques may also be employed, such as using biotin modified
nucleotides for introduction into a polynucleotide. The
biotin then serves as the site for binding to avidin or
antibodies, which may be labeled with a wide variety of

labels, such as radionuclides, fluorescers, enzymes, or the
like. Alternatively, antibodies may be employed which can
recognize specific duplexes, including DNA duplexes, RNA
duplexes, DNA-RNA hybrid duplexes, or DNA-protein duplexes.

5   The antibodies in turn may be labeled and the assay carried
out where the duplex is bound to a surface, so that upon the
formation of duplex on the surface, the presence of antibody
bound to the duplex can be detected. The use of probes to the
novel anti-sense RNA may be carried out in any conventional

10   techniques such as nucleic acid hybridization, plus and minus
screening, recombinational probing, hybrid released
translation (HRT), and hybrid arrested translation (HART).
This also includes amplification techniques such as polymerase
chain reaction (PCR).

15   Diagnostic kits which also test for the qualitative or
quantitative presence of other markers are also contemplated.
Diagnosis or prognosis may depend on the combination of
multiple indications used as markers. Thus, kits may test for
combinations of markers. See, e.g., Viallet, et al. (1989)

20   Progress in Growth Factor Res. 1:89-97.

VIII. Therapeutic Utility
     This invention provides reagents with significant
therapeutic value. The IL-1δ or IL-1ε (naturally occurring or

25   recombinant), fragments thereof, mutein agonists and
antagonists, and antibodies, along with compounds identified
as having binding affinity to the interleukin or its receptor
or antibodies, should be useful in the treatment of conditions
exhibiting abnormal expression of the interleukin. Such

30   abnormality will typically be manifested by immunological
disorders. Additionally, this invention should provide
therapeutic value in various diseases or disorders associated
with abnormal expression or abnormal triggering of response to
the interleukin. The mouse IL-γ has been suggested to be

involved in tumors, allergies, and infectious diseases, e.g.,
pulmonary tuberculosis, leprosy, fulminant hepatitis, and
viral infections, such as HIV. The IL-1δ and/or IL-1ε or
antagonist may have similar function, and the signaling has
5   been shown to involve NFκB (nuclear factor κB).

        In addition, the dendritic cell expression profile shows
human IL-1γ primarily expressed in activated dendritic cells.
Activated dendritic cells are also a major producer of IL-12,
and it is thought that this dendritic cell produced IL-12
10   plays a major role in directing a Th1 type response. The
combination of IL-1γ and IL-12 should be extremely potent in
inducing IFN-γ, suggesting that IL-1δ or IL-1ε, or antagonists
thereof, may have similar function. It is possible that the
combination of pro-inflammatory cytokines under certain
15   circumstances could lead to septic shock. An antagonist,
mutein or antibody, could prove very useful in this situation.
See Rich (ed.) Clinical Immunology: Principles and Practice,
Mosby.

        Additionally, IL-1δ or IL-1ε being homologous members of
20   the IL-1 family likely play a role in modulating of local and
systemic inflammatory processes (see, Durum, et al. (1985)
Ann. Rev. Immunol. 3:263-287), through the enhancement of
blood flow, induction of chemoattractants, and the enhancement
and adherence of adhesion molecules resulting in the
25   accumulation of inflammatory cells such as macrophages and
neutrophils at the site of inflammation. Additionally, it is
likely that IL-1δ or IL-1ε induce fibroblast growth and may
play a role in contributing to the pathogenesis of chronic
inflammation, as in rheumatoid arthritis or periodontal
30   disease.

        IL-1δ or IL-1ε are also likely to play a role in systemic
inflammatory reactions, such as fever, hypoglycemia, the acute
phase response of the liver, reduced plasma iron and zinc, and
increased plasma copper. A systemic reaction such as septic

shock involves vasodilation, due to IL-1, most likely in combination with other cytokines, including, e.g., TNF, IFN-γ, and leukemia inhibitory factor (LIF). The newly described IL-1δ or IL-1ε are also likely to be similarly involved.

5   T helper cells mediate effector functions in infectious, allergic, or autoimmune diseases through production of cytokines. CD4+ T cells can be divided into Th1 and Th2 subsets on the basis of their cytokine profile upon antigen stimulation. Evidence has recently been obtained that Th1 and

10   Th2 cells differ in responsiveness and receptor expression for IL-1 family molecules. See, e.g., Robinson, et al. (1997) Immunity 7:571-581. Whereas Th1 cells respond to IL-1γ, Th2 cells respond to IL-1α. This differential responsiveness between Th1 and Th2 cells to IL-1γ and IL-1α, respectively,

15   may have profound implications for regulation of ongoing Th cell responses. The novel IL-1 molecules described here could play a similar role in either supporting a Th1 or Th2 response, depending on the presence or absence of their cognate IL-1 receptors on the cell surface of these immune

20   cells; e.g., IL-1RD4 (ST2) is an orphan IL-1-like receptor exclusively expressed on the Th2 subset. See, e.g., Lohning, et al. (1998) Proc. Nat'l Acad. Sci. USA 95:6930-6935; and USSN 09/040,714, which are incorporated herein by reference. The expression profile of the novel IL-1 proteins here

25   described (in particular IL-1ε) indicates that IL-1ε could be the ligand for IL-1RD4 and, as such, could be important for Th2 effector function.

      In the following, directed to IL-1δ, similar substitution of IL-1ε may be appropriate. Recombinant IL-1δ, mutein

30   agonists or antagonists, or IL-1δ antibodies can be purified and then administered to a patient. These reagents can be combined for therapeutic use with additional active ingredients, e.g., in conventional pharmaceutically acceptable carriers or diluents, along with physiologically innocuous

stabilizers and excipients. These combinations can be
sterile, e.g., filtered, and placed into dosage forms as by
lyophilization in dosage vials or storage in stabilized
aqueous preparations. This invention also contemplates use of
5 antibodies or binding fragments thereof which are not
complement binding.

Receptor screening using IL-1δ or fragments thereof can
be performed to identify molecules having binding affinity to
the interleukin. Subsequent biological assays can then be
10 utilized to determine if a receptor can provide competitive
binding, which can block intrinsic stimulating activity.
Receptor fragments can be used as a blocker or antagonist in
that it blocks the activity of IL-1δ. Likewise, a compound
having intrinsic stimulating activity can activate the
15 receptor and is thus an agonist in that it simulates the
activity of IL-1δ. This invention further contemplates the
therapeutic use of antibodies to IL-1δ as antagonists.

The quantities of reagents necessary for effective
therapy will depend upon many different factors, including
20 means of administration, target site, physiological state of
the patient, and other medicants administered. Thus,
treatment dosages should be titrated to optimize safety and
efficacy. Typically, dosages used in vitro may provide useful
guidance in the amounts useful for in situ administration of
25 these reagents. Animal testing of effective doses for
treatment of particular disorders will provide further
predictive indication of human dosage. Various considerations
are described, e.g., in Gilman, et al. (eds 1990) Goodman and
Gilman's: The Pharmacological Bases of Therapeutics, 8th Ed.,
30 Pergamon Press; and Remington's Pharmaceutical Sciences,
(current ed.), Mack Publishing Co., Easton, Penn.; each of
which is hereby incorporated herein by reference. Methods for
administration are discussed therein and below, e.g., for
oral, intravenous, intraperitoneal, or intramuscular

administration, transdermal diffusion, and others.
Pharmaceutically acceptable carriers will include water,
saline, buffers, and other compounds described, e.g., in the
Merck Index, Merck & Co., Rahway, New Jersey. Because of the
5   likely high affinity binding between an IL-1δ and its
receptors, low dosages of these reagents would be initially
expected to be effective. And the signaling pathway suggests
extremely low amounts of ligand may have effect. Thus, dosage
ranges would ordinarily be expected to be in amounts lower
10   than 1 mM concentrations, typically less than about 10 μM
concentrations, usually less than about 100 nM, preferably
less than about 10 pM (picomolar), and most preferably less
than about 1 fM (femtomolar), with an appropriate carrier.
Slow release formulations, or slow release apparatus will
15   often be utilized for continuous administration.

     IL-1δ fragments thereof, and antibodies or its fragments,
antagonists, and agonists, may be administered directly to the
host to be treated or, depending on the size of the compounds,
it may be desirable to conjugate them to carrier proteins such
20   as ovalbumin or serum albumin prior to their administration.
Therapeutic formulations may be administered in any
conventional dosage formulation. While it is possible for the
active ingredient to be administered alone, it is preferable
to present it as a pharmaceutical formulation. Formulations
25   comprise at least one active ingredient, as defined above,
together with one or more acceptable carriers thereof. Each
carrier must be both pharmaceutically and physiologically
acceptable in the sense of being compatible with the other
ingredients and not injurious to the patient. Formulations
30   include those suitable for oral, rectal, nasal, or parenteral
(including subcutaneous, intramuscular, intravenous and
intradermal) administration. The formulations may
conveniently be presented in unit dosage form and may be
prepared by any methods well known in the art of pharmacy.

See, e.g., Gilman, et al. (eds. 1990) <u>Goodman and Gilman's:</u>
<u>The Pharmacological Bases of Therapeutics</u>, 8th Ed., Pergamon
Press; and <u>Remington's Pharmaceutical Sciences</u>, 17th ed.
(1990), Mack Publishing Co., Easton, Penn.; Avis, et al. (eds.
5   1993) <u>Pharmaceutical Dosage Forms: Parenteral Medications</u>
Dekker, NY; Lieberman, et al. (eds. 1990) <u>Pharmaceutical</u>
<u>Dosage Forms: Tablets</u> Dekker, NY; and Lieberman, et al. (eds.
1990) <u>Pharmaceutical Dosage Forms: Disperse Systems</u> Dekker,
NY.

10   Another therapeutic approach included within the
invention involves direct administration of reagents or
compositions by any conventional administration techniques
(e.g., but not restricted to local injection, inhalation, or
administered systemically), to the subject with an
15   inflammatory disorder. The reagent, formulation or
composition may also be targeted to specific cells or
receptors by any of the methods described herein. The actual
dosage of reagent, formulation or composition that modulates
an inflammatory disorder depends on many factors, including
20   the size and health of an organism, however one of one of
ordinary skill in the art can use the following teachings
describing the methods and techniques for determining clinical
dosages. See, e.g., Spilker (1984) <u>Guide to Clinical Studies</u>
<u>and Developing Protocols</u>, Raven Press, New York, particularly
25   pages 7-13, 54-60; Spilker (1991) <u>Guide to Clinical Trials</u>,
Raven Press, New York, especially pages 93-101; Craig and
Stitzel (eds. 1986) <u>Modern Pharmacology</u> 2d ed., Little, Brown,
Boston, especially pages 127-33; Speight (ed. 1987) <u>Avery's</u>
<u>Drug Treatment: Principles and Practice of Clinical</u>
30   <u>Pharmacology and Therapeutics</u>, 3d ed., Williams and Wilkins,
Baltimore, pages 50-56; and Tallarida, et al. (1988)
<u>Principles in General Pharmacology</u>, Springer-Verlag, New York,
pages 18-20; which describe how to determine the appropriate
dosage; but, generally, in the range of about between 0.5

ng/ml and 500 µg/ml inclusive final concentration are
administered per day to an adult in a pharmaceutically-
acceptable carrier. The therapy of this invention may be
combined with or used in association with other therapeutic
5   agents, particularly agonists or antagonists of other IL-1
family members.

   The IL-R6 receptor subunit forms the baasis for a good
candidate for an antagonist drug development (i.e., humanized
anti-human IL-1R6 antibodies). IL-1R6 seems to be mainly
10 expressed on resident epithelial cells, constituting the ports
of entry to the body's internal milieu. IL-1R6 may form an
integral part of the innate immune defense against exposure to
external micro-organisms. IL-1R6's ligand, IL-1ε, activates
NFκB, as do many pro-inflammatory mediators (e.g., LPS, IL-1s,
15 and TNFs). NFκB is a transcription factor widely involved in
the induction of expression of inflammatory mediators, such as
numerous cytokines, chemokines, and their adhesion molecules.
The increased expression of IL-1ε and IL-1R6 in chronically
inflamed skin (e.g., psoriasis) points to the possible
20 clinical relevance of this R6 signaling system. The fact that
the other component of this system (IL-1δ) acts as a very
potent antagonist might prove to have clinical use.

   IX.   Receptors
25   The description of the IL-1δ ligand herein provides means
to identify a receptor, as described above. Such receptor
should bind specifically to the IL-1δ with reasonably high
affinity. Various constructs are made available which allow
either labeling of the IL-1δ to detect its receptor. For
30 example, directly labeling IL-1δ, fusing onto it markers for
secondary labeling, e.g., FLAG or other epitope tags, etc.,
will allow detection of receptor. This can be histological,
as an affinity method for biochemical purification, or
labeling or selection in an expression cloning approach. A

two-hybrid selection system may also be applied making
appropriate constructs with the available IL-1δ sequences.
See, e.g., Fields and Song (1989) Nature 340:245-246.
Typically, a cytokine will bind to its receptor at a Kd of at
5   least about 30 μM, preferably at least about 10 μM, and more
preferably at least about 3 μM or better; including 1 μM, 300
nM, 100 nM, 30 nM, etc.

     Generally, descriptions of how to make IL-1δ will be
analogously applicable to embodiments directed to IL-1ε
10   reagents and compositions.

     Interleukin-1 (IL-1) is of utmost importance in the host
responses to immunological challenges. IL-1 ligand as well as
IL-1 receptor (R) isoforms affect and tightly control IL-1's
net biological activity. This application, with earlier
15   filings, reports identification of novel IL-1 ligands based on
homology to IL-1 receptor antagonist (IL-1RA), which are
termed IL-1δ and IL-1ε. RNA expression data showed that these
IL-1s are highly expressed in embryonic tissue and tissues
containing epithelial cells (e.g., skin, lung, and stomach).
20   With respect to the skin, keratinocytes are the main producers
of IL-1δ/ε. IL-1δ/ε proteins lack a classical leader sequence
(as does secreted IL-1RA) or a clear pro-form (as do IL-
1α/β/γ), but do get secreted in the supernatant of transiently
transfected cells. Functionally, using nuclear factor κB
25   (NFκB)-Luciferase reporter assays, it has been demonstrated
that IL-1δ/ε proteins do not initiate a response through the
classical pathway of other IL-1Rs, which confer responsiveness
to either IL-1α/β (e.g., IL-1RI/IL-1R accessory protein) or
IL-18/IL-1γ (e.g., IL-1R related protein 1/IL-1R accessory
30   protein-like). However, when testing the orphan IL-1R-like
molecules T1/ST2 and IL-1R related protein 2 (IL-1Rrp2), it
was reproducibly found to activate NFκB upon stimulation with
IL-1ε, but not IL-1δ. IL-1δ, showing the highest similarity to

IL-1RA, on the other hand, specifically inhibited this IL-
1Rrp2 response to IL-1ε. IL-1δ is, in fact, a highly potent
antagonist for IL-1Rrp2, as the effective ratio between IL-1δ
and IL-1ε is 3 orders of magnitude less than for IL-1R1 (e.g.,
5   IL-1RA:IL-1α).

IL-1δ and ε were identified computationally:
Computational analyses led to the discovery of two novel IL-1
ligands: IL-1δ and IL-1ε. In short, the search strategies were
based on homology and HMMer searches. A blast search with
10   mouse IL-1RA revealed a public EST containing the entire open
reading frame of mouse IL-1δ. Subsequently, the entire human
IL-1δ sequence was found as part of a proprietary EST. Using
mouse IL-1δ as a bait also led to the finding of a public EST
containing a partial sequence of mouse IL-1ε, which was
15   extended in the 5' direction to obtain the full length
sequence. Another proprietary EST containing the full length
human IL-1ε sequence was ultimately found by a HMMer search
using a PFAM alignment of IL-1α, IL-1β, and IL-1RA.

IL-1δ and IL-1ε are highly expressed in embryonic tissue
20   and in epithelial cells: Northern blot analyses show that the
novel IL-1 ligands are expressed in embryonic tissue, and
tissues containing epithelial cells (e.g., stomach and skin).
Expression in lung tissue appeared unique to IL-1δ. It should
also be noted that IL-1δ mRNA analysis shows the presence of
25   multiple variants (as does IL-1RA). Hannum, et al. (1990)
Nature 343:336-340; Eisenberg, et al. (1990) Nature 343:341-
346; Haskill, et al. (1991) Proc. Nat'l Acad. Sci. USA
88:3681-3685; Muzio, et al. (1995) J. Exp. Med. 182:623-628;
and Weissbach, et al. (1998) Biochem. Biophys. Res. Commun.
30   244:91-95. Additional studies, based on quantitative PCR,
revealed that in skin, keratinocytes and langerhans cells, but
not fibroblasts or skin-homing T cells, are the main producers
of IL-1δ and IL-1ε.

IL-1δ and IL-1ε proteins are secreted: The novel IL-1 ligands are secreted as determined by their presence in the supernatant of transiently transfected 293-T cells. However, they do not have a classical leader sequence (as does secreted

5   IL-1RA; Eisenberg, et al. (1990) <u>Nature</u> 343:341-346), nor do they have a clear pro-form (as do IL-1α/β/γ; March, et al. (1985) <u>Nature</u> 315:641-647; Okamura, et al. (1995) <u>Nature</u> 378:88-91; and Ushio, et al. (1996) <u>J. Immunol.</u> 156:4274-4279.

IL-1δ and IL-1ε do not activate NFkB through classical IL-

10   1Rs: The ability of the novel IL-1s to initiate IL-1R-mediated signaling was studied via an NFκB-dependent reporter assay using ligand-stimulated Jurkat T cells transiently transfected with different pairs of IL-1Rs. The R1/R3 combination, being responsive to IL-1α and IL-1β (Greenfeder,

15   et al. (1995) <u>J. Biol. Chem.</u> 270:13757-13765; and Cullinan, et al. (1998) <u>J. Immunol.</u> 161:5614-5620, did not generate a response to IL-1δ or IL-1ε. Also, the R5/R7 combination, responsible for the response to IL-18/IL-1γ (Born, et al. (1998) <u>J. Biol. Chem.</u> 273:29445-29450), did not result in

20   signaling upon addition of the novel IL-1 ligands. The next step was to test the orphan IL-1R-like molecules, IL-1R4 and IL-1R6 (classified as potential ligand-binding receptors, based on their homology to IL-1R1) in combination with various other IL-1R-like molecules, e.g., IL-1R3, IL-1R7, IL-1R9 and

25   IL-1R10 (classified as potential signaling receptors, based on their homology to IL-1R3) for their responsiveness to IL-1δ and IL-1ε. These data consistently showed an IL-1R6-mediated activation of NFκB upon stimulation with IL-1ε, but not IL-1δ or the mock control.

30   IL-1δ specifically and very potently antagonizes the IL-1R6 response to IL-1ε: In line with the striking similarity between IL-1δ and IL-1RA, the possibility of IL-1δ being an antagonist of IL-1 responses was tested to distinguish from it

being an IL-1 agonist. Using the same reporter assay, it was
shown that IL-1RA, but not IL-1δ, is able to antagonize the
IL-1R1-mediated activation of NFκB upon stimulation with IL-
1α. Reciprocally, IL-1δ, but not IL-1RA, is able to

5   antagonize the IL-1R6-mediated activation of NFκB upon
stimulation with IL-1ε. One can conclude that IL-1RA shows a
50% inhibition of the IL-1R1 response at about a 1000-fold
excess over IL-1α, whereas IL-1δ results in a similar
inhibition of the IL-1R6 response at concentrations similar to

10   or even less than IL-1ε.

The current application describes the discovery of two
novel members of the IL-1 family. This is in extension to
recent reports on new IL-1 family members (Mulero, et al.
(1999) Biochem. Biophys. Res. Commun. 263:702-706; Smith, et

15   al. (2000) J. Biol. Chem. 275:1169-1175; and USSN 09/130,972.
The present report is unique in several aspects. First, it is
shown that these novel IL-1s have a restricted expression
pattern, with a high expression in epithelial cells. Second,
it has been possible to express and purify adenovirus-derived

20   human IL-1δ and IL-1ε proteins which show function, e.g., they
affect IL-1 signaling mediated through the orphan IL-1R-like
molecule, IL-1R6 (e.g., IL-1Rrp2). Third, the novel IL-1
ligands together with IL-1R6, constitute a novel IL-1R system
with IL-1ε acting as an agonist and IL-1δ as an antagonist in

25   analogy to the IL-1R1 system with IL-1α and IL-1β acting as
agonists and IL-1RA as an antagonist.

A structurally-aided alignment of the novel IL-1s and the
classical IL-1s indicates the conservation of core 12 β-
strands, making up a β-trefoil structure. At the sequence

30   level, IL-1δ and IL-1RA show a high degree of similarity,
which is also evident from the evolutionary tree analysis.
With respect to IL-1ε, it is interesting to note that several
public mouse ESTs exist, mostly derived from tongue

epithelium, with only slight variations relative to the
presented IL-1ε sequence. In addition, FIL-1ε (Smith, et al.
(2000) J. Biol. Chem. 275:1169-1175) also shows a very high
similarity to the human IL-1ε sequence provided (e.g., 51%).

5   How these IL-1ε variants are generated and their biological
significance remain unclear. The IL-1δ and IL-1ε genes cluster
together and are localized to the same region of chromosome 2q
as the IL-1α, IL-1β, and IL-1RA genes (see also Mulero, et al.
(1999) Biochem. Biophys. Res. Commun. 263:702-706; and Smith,

10 et al. (2000) J. Biol. Chem. 275:1169-1175), stressing the
notion that these IL-1 family members are all derived from a
common ancestor.

    IL-1δ and IL-1ε have a strong expression in embryonic
development, and in tissues such as stomach, lung and skin.

15 Lung tissue only showed IL-1δ mRNA expression, although at PCR
level, expression of both isoforms can be detected in lung-
derived cDNAs. The predominant messages for IL-1δ are 1.2 and
2.6 kb for stomach and skin tissues and 2.0 kb for lung
tissue. It is of interest to note that IL-1δ mRNA in lung

20 tissue appears to lack an exon relative to other tissues.
Smith, et al. (2000) J. Biol. Chem. 275:1169-1175. Therefore,
the different messages might in fact reflect different
(tissue-specific) splice variants, as seen for IL-1RA (Hannum,
et al. (1990) Nature 343:336-340; Eisenberg, et al. (1990)

25 Nature 343:341-346; Haskill, et al. (1991) Proc. Nat'l Acad.
Sci. USA 88:3681-3685; Muzio, et al. (1995) J. Exp. Med.
182:623-628; and Weissbach, et al. (1998) Biochem. Biophys.
Res. Commun. 244:91-95. Tissue distribution and presence of
differently sized messages of IL-1RA was strikingly similar to

30 that of IL-1δ. Expression of IL-1δ variants might be, in line
with IL-1RA, under the control of different promotors
(Butcher, et al. (1994) J. Immunol. 153:701-711). A more
detailed look at human skin, using quantitative PCR analysis
of a panel of first strand cDNAs derived from various skin-

specific cell types, pointed to keratinocytes, and not
fibroblasts or skin-homing T cells, as the major source of
these IL-1s. Preliminary in situ hybridisation data using
mouse tissue sections confirmed that cells of epithelial
5   origin, such as the parietal and chief cells in stomach, and
basal keratinocytes in skin are the predominant cellular
sources of these IL-1s. Because of their presence in
epithelial barriers of the body (e.g., skin, digestive and
respiratory tracts), one could speculate that IL-1δ and IL-1ε
10   fulfill similar roles as their known family members (e.g., IL-
1α and IL-1β) to promote a response to injury or infection.
Dinarello (1994) Eur. Cytokine Netw. 5:517-531; and Kupper
(1989) [published erratum appears in (1989) Arch. Dermatol.
125(12):1643] Arch.Dermatol. 125:1406-1412. Activities
15   crucial to such a response include control of the
proliferative status of epithelial cells, tissue remodelling
(via matrix metalloproteinases) and production of pro-
inflammatory molecules (e.g., cytokines, chemokines, their
receptors and adhesion molecules). In non-cornified stomach
20   epithelium, having a high expression of IL-1δ and IL-1ε
relative to IL-1α and IL-1β, the novel IL-1s might display
more unique functions, e.g., promoting cross-talk between
stomach epithelial cells and the underlying (sub)mucosa-
associated immune cells.

25   IL-1s are thought to function intracellularly under
certain conditions, as suggested by the intracellular
localization of pro-IL-1α (which is biologically active; see,
Mosley, et al. (1987) J. Biol. Chem. 262:2941-2944) and
intracellular IL-1RA isoforms in several epithelia (Dinarello
30   (1994) Eur. Cytokine Netw. 5:517-531; and Arend (1993)
Adv.Immunol. 54:167-227). The observation that the novel IL-
1s lack a signal peptide as well as a clear pro-form, leads
one to suppose that these new IL-1 proteins may be retained
intracellularly. However, monitoring the presence of C-

terminally tagged versions of IL-1δ and IL-1ε in the
supernatants and lysates of transfected cells showed that
these molecules do get secreted efficiently. The presence of
alternate splice forms of IL-1δ leaves open the possibility

5   that other forms of IL-1δ do exist. Such would agree with
Thomassen, et al. (1999) Cytokine 11:389-399, who showed that
the novel IL-1R-like molecule SIGIRR (e.g., IL-1R8) expresses
as a glycosylated transmembrane protein without having an
obvious signal peptide, and concluded that this probably

10 reflects limitations in our understanding of extracellular
protein transport.
        To study whether the novel IL-1s function like the
classical IL-1s, the genes were expressed and purified as
adenovirus-derived human IL-1δ and IL-1ε, and tested for their

15 capacity to initiate IL-1 signaling, with an NFκB reporter
assay as a read-out. The observation that the classical IL-1R
pairs IL-1R1/3 and IL-1R5/7 do not respond to these new
protein preparations might be explained by the fact that
receptor-ligand combinations within the IL-1 system are very

20 specific. Therefore, IL-1R4 and IL-1R6 were tested in
combination with various other IL-1R-like molecules. These
studies consistently showed the usage of IL-1R6 by IL-1ε, but
not IL-1δ, to activate NFκB in Jurkat cells. Even IL-1R6
single transfections showed this response. The IL-1 system,

25 as understood today, typically requires two receptors, one
ligand-binding receptor and one signaling receptor, to get an
IL-1 response. Since IL-1R6 is very homologous to IL-1R1
(Lovenberg, et al. (1996) J. Neuroimmunol. 70:113-122), a
ligand-binding type of receptor, it was supposed that Jurkat

30 cells endogenously express a second signaling type of receptor
that can pair with IL-1R6 in the presence of IL-1ε. By PCT
analysis, the following IL-1R-like molecules are expressed by
untransfected Jurkat cells: IL-1R3, IL-1R4, IL-1R8 (e.g.,
SIGIRR), IL-1R9, and IL-. Co-transfection of IL-1R6 with IL-

1R3, IL-1R9, or IL-1R10 does not potentiate the response to
IL-1ε relative to IL-1R6 single transfectants. In addition,
studies by others using IL-1R1 chimaeras and an IL-1α-mediated
activation of NFκB as a read-out do not seem to favor the
5   combination of IL-1R6 and IL-1R8 to mediate an IL-1 response.
Thomassen, et al. (1999) Cytokine 11:389-399. The search for
the additional IL-1ε receptor(s) is currently ongoing.

IL-1δ is most closely related to IL-1RA, and, like IL-
1RA, lacks the loop between the fourth and fifth β-strands
10 which is typical for IL-1 agonists: IL-1α, IL-1β, IL-1γ, and
IL-1ε. Insertion of the loop amino acids QG...SN of IL-1β
confers, in fact, agonist activity to IL-1RA. Greenfeder, et
al. (1995) J. Biol. Chem. 270:22460-22466. In extension of
the striking similarity between IL-1δ and IL-1RA, it was
15 hypothesized that IL-1δ might act as an antagonist.
Experimentally, IL-1δ turned out to be a very specific and
potent antagonist. It still antagonizes the IL-1R6-mediated
response to IL-1ε at a ratio of IL-1δ:IL-1ε being less than 1.
The potency of IL-1RA to antagonize the R1-response to IL-1α/β
20 is about 3 orders of magnitude less. The observation by
others (Smith, et al. (2000) J. Biol. Chem. 275:1169-1175)
that their IL-1δ and IL-1ε do not bind to IL-1R6 is in our
opinion not contradictory to our findings. Binding studies
using partially purified IL-1δ and IL-1ε proteins from
25 conditioned medium of transfected cells and an Fc fusion of
IL-1R6 might not be sensitive enough to show binding to these
new IL-1s. Moreover, the second receptor might actually be
needed for affinity conversion and for binding to become
detectable.

30 Expression of human IL-1R6 is limited when compared to
human IL-1R1 or R3, which are expressed ubiquitously, and is
restricted to lung epithelium and brain vasculature.
Lovenberg, et al. (1996) J. Neuroimmunol. 70:113-122.

Expression of IL-1R6 was found in monocytes and in skin-derived keratinocytes and fibroblasts, indicating that these cell types might be IL-1ε responding cells. Preliminary data indicate that the expression of IL-1δ, IL-1ε, and IL-1R6 are

5   increased in lesional psoriasis skin samples relative to normal control skin samples. These data are being followed up, but indicate a clinical relevance of these novel IL-1 ligands.

Taken together, IL-1δ, IL-1ε, and IL-1R6 probably

10   constitute a system on their own, in analogy to IL-1α/β/ra and IL-1R1. This IL-1R6 system, present in epithelial barriers of the body, is, as a result of the co-expression of IL-1δ and IL-1ε, likely to have a default off-state. Perturbation of homeostasis, however, can shift this balance to a protective

15   IL-1ε-mediated inflammatory or proliferative response.

The broad scope of this invention is best understood with reference to the following examples, which are not intended to limit the inventions to the specific embodiments.

The invention illustratively described herein suitably

20   may be practiced in the absence of any element or elements, limitation or limitations which is not specifically disclosed herein. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms an

25   expression of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible with the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed

30   by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those of ordinary skill in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

EXAMPLES

I.   General Methods

5   Some of the standard methods are described or referenced,
e.g., in Maniatis, et al. (1982) Molecular Cloning, A
Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring
Harbor Press; Sambrook, et al. (1989) Molecular Cloning: A
Laboratory Manual, (2d ed.), vols 1-3, CSH Press, NY;
10   Ausubel, et al., Biology, Greene Publishing Associates,
Brooklyn, NY; or Ausubel, et al. (1987 and Supplements)
Current Protocols in Molecular Biology, Greene/Wiley, New
York. Methods for protein purification include such methods
as ammonium sulfate precipitation, column chromatography,
15   electrophoresis, centrifugation, crystallization, and others.
See, e.g., Ausubel, et al. (1987 and periodic supplements);
Deutscher (1990) "Guide to Protein Purification" in Meth.
Enzymol., vol. 182, and other volumes in this series; and
manufacturer's literature on use of protein purification
20   products, e.g., Pharmacia, Piscataway, N.J., or Bio-Rad,
Richmond, CA. Combination with recombinant techniques allow
fusion to appropriate segments, e.g., to a FLAG sequence or an
equivalent which can be fused via a protease-removable
sequence. See, e.g., Hochuli (1989) Chemische Industrie
25   12:69-70; Hochuli (1990) "Purification of Recombinant Proteins
with Metal Chelate Absorbent" in Setlow (ed.) Genetic
Engineering, Principle and Methods 12:87-98, Plenum Press,
N.Y.; and Crowe, et al. (1992) QIAexpress: The High Level
Expression & Protein Purification System QIAGEN, Inc.,
30   Chatsworth, CA.
         Computer sequence analysis is performed, e.g., using
available software programs, including those from the GCG (U.
Wisconsin) and GenBank sources. Public sequence databases
were also used, e.g., from GenBank and others.

        Many techniques applicable to IL-4 and IL-10 may be
applied to IL-1δ and/or IL-1ε, as described, e.g., in U.S.
Patent No. 5,017,691 (IL-4), USSN 07/453,951 (IL-10), USSN
08/110,683 (IL-10 receptor), and USSN 09/130,972 (rodnet IL-1δ
5   and rodent and primate IL-1ε) each of which is incorporated
herein by reference for all purposes.   Biological Reagents
and Cell culture: Recombinant human IL-1α, IL-1β, IL-4, and
IFNγ were provided by R&D Systems. Recombinant human IL-18/IL-
1γ, IL-1RA and IL-10 were produced at DNAX.   The Q293 and 293-T
10 cell lines were maintained in DMEM supplemented with 5% fetal
bovine serum, 0.3 mg/ml L-glutamine, 100 U/ml penicillin G,
and 100 μg/ml streptomycin (Life Technologies, Inc.).   Human
primary epidermal keratinocytes and dermal fibroblasts
(Clonetics) were cultured in specialized growth media
15 according to the supplier's recommendations. The Jurkat E6.1
cell line was maintained in RPMI 1640 medium supplemented with
10% fetal bovine serum, glutamine, and antibiotics.

II. Amplification of IL-1δ or IL-1ε fragment by PCR
20   Cloning of human and mouse IL-1δ and IL-1ε: See USSN
09/130,972, which is incorporated herein by reference.
Briefly, searching public expressed sequence tag (EST)
database with the common portion of murine IL-1RA revealed EST
mb49b11.r1 (Genbank accession # W08205), which contained the
25 full-length sequence of a novel rodent IL-1-like molecule,
designated IL-1δ. Using the mouse sequence as a query, a
human EST derived from RNA from bronchial smooth muscle cells
was found in a proprietary database containing the full-length
open-reading frame of a human homologue of mouse IL-1δ.   The
30 same query sequence revealed an additional EST mi08c10.r1
(Genbank accession # AA030324), which contained partial
sequence of a second novel IL-1-like molecule, designated IL-
1ε. The full-length sequence of murine IL-1ε was obtained by

extending the 5`sequence by RACE-PCR on murine 17 d old embryo
Marathon-Ready library cDNA (Clontech). Finally, a HMMer
(http://hmmer.wustl.edu/) search using a PFAM alignment of IL-
1α, IL-1β and IL-1RA (http://pfam.wustl.edu/) revealed an EST
5   derived from RNA from epithelial cells in a second proprietary
database, which contained the full-length open-reading frame
of the human IL-1ε.

     The novel IL-1 sequences were aligned to the published
IL-1 sequences using CLUSTALW (Thompson, et al. (1994) <u>Nucleic</u>
10   <u>Acids Res.</u> 22:4673-4680), guided by tertiary structures and
predicted secondary structures (with a consensus derived from
several algorithms at
http://circinus.ebi.ac.uk:8081/submit.html), and fine-tuning
by eye. Conserved alignment patterns were drawn by Consensus
15   (http://www.bork.emblheidelberg.de/Alignment/ consensus.html).
Evolutionary tree analysis was performed using a neighbor-
joining algorithm and viewed using TreeView 1.5.

     The IL-1 ligands appear to contain twelve conserved β-
strands, particularly with the human IL-1β and IL-1RA. See,
20   e.g., Vigers, et al. (1997) <u>Nature</u> 386:190-194; and Schreuder,
et al. (1997) <u>Nature</u> 386:194-200. These are based, in part,
on predicted secondary structures. Particular residues of
human IL-1β which interact with IL-1R1 (sites A and B) have
been described, and reflect on residues of other family
25   members which structurally are predicted to interact with
receptor subunits. See Ju, et al. (1991) <u>Proc. Nat'l Acad.</u>
<u>Sci. USA</u> 88:2658-2662.

     There are various methods of isolating the DNA sequences
encoding IL-1δ and IL-1ε proteins. For example, DNA is
30   isolated from a genomic or cDNA library using labeled
oligonucleotide probes having sequences identical or
complementary to the sequences disclosed herein. Full-length
probes may be used, or oligonucleotide probes may be generated
by comparison of the sequences disclosed. Such probes can be

used directly in hybridization assays to isolate DNA encoding
thymokine proteins, or probes can be designed for use in
amplification techniques such as PCR, for the isolation of DNA
encoding IL-1δ and IL-1ε proteins.

5   Various methods of amplifying target sequences, such as
the polymerase chain reaction, can also be used to prepare DNA
encoding IL-1δ and IL-1ε proteins. Polymerase chain reaction
(PCR) technology is used to amplify such nucleic acid
sequences directly from mRNA, from cDNA, and from genomic

10   libraries or cDNA libraries. The isolated sequences encoding
IL-1δ or IL-1ε proteins may also be used as templates for PCR
amplification.

        In PCR techniques, oligonucleotide primers complementary
to two 5' regions in the DNA region to be amplified are

15   synthesized. The polymerase chain reaction is then carried
out using the two primers. See Innis et al. (current eds.)
PCR Protocols: A Guide to Methods and Applications Academic
Press, San Diego, CA. Primers can be selected to amplify the
entire regions encoding a full-length IL-1δ or IL-1ε proteins

20   or to amplify smaller DNA segments as desired. Once such
regions are PCR-amplified, they can be sequenced and
oligonucleotide probes can be prepared from sequence obtained
using standard techniques. These probes can then be used to
isolate DNA's encoding tIL-1δ or IL-1ε proteins.

25   Oligonucleotides for use as probes are chemically
synthesized according to the solid phase phosphoramidite
triester method first described by Beaucage and Carruthers
(1983) Tetrahedron Lett. 22(20): 1859-1862, or using an
automated synthesizer, as described in Needham-VanDevanter et

30   al. (1984) Nucleic Acids Res. 12: 6159-6168. Purification of
oligonucleotides is performed e.g., by native acrylamide gel
electrophoresis or by anion-exchange HPLC as described in
Pearson and Regnier (1983) J. Chrom. 255: 137-149. The
sequence of the synthetic oligonucleotide can be verified

using the chemical degradation method of Maxam and Gilbert in
Grossman and Moldave (eds. 1980) <u>Methods in Enzymology</u>
65:499-560 Academic Press, New York.

5   The peptide segments, along with comparison to homologous
genes, can also be used to produce appropriate
oligonucleotides to screen a library. The genetic code can be
used to select appropriate oligonucleotides useful as probes
for screening. In combination with polymerase chain reaction
(PCR) techniques, synthetic oligonucleotides will be useful in
10   selecting desired clones from a library

     Complementary sequences will also be used as probes or
primers. Based upon identification of the likely amino
terminus, other peptides should be particularly useful, e.g.,
coupled with anchored vector or poly-A complementary PCR
15   techniques or with complementary DNA of other peptides.

     To identify a homologous IL-1δ or IL-1ε proteins,
degenerate oligonucleotides are designed which corresponded to
conserved regions among known IL-1 family members. The
primers are used for polymerase chain reactions on mouse
20   genomic DNA followed by subcloning the PCR products using
restriction sites placed at the 5' ends of the primers,
picking individual E. coli colonies carrying these subcloned
inserts, and using a combination of random sequencing and
hybridization analysis to eliminate known IL-1 family members.

25   Subsequently, PCR products are gel-purified, digested
with appropriate restriction enzymes, gel-purified again, and
subcloned in the Bluescript vector (Stratagene, San Diego,
CA). Bacterial colonies carrying individual subclones are
picked into 96 well microtiter plates, and multiple replicas
30   are prepared by plating the cells onto nitrocellulose. The
replicate filters are hybridized to probes representing known
members of the IL-1 family, and DNA is prepared from non-
hybridizing colonies for sequence analysis.

　　　　Two appropriate forward and reverse primers are selected
using the sequences supplied herein (see SEQ ID Nos:1 and 3)
and common knowledge. See, e.g., Innis, et al. (current eds.)
PCR Protocols: A Guide to Methods and Applications Academic

5　　Press, San Diego, CA; and Dieffenbach and Dveksler (current
eds.) PCR Primer: A Laboratory Manual Cold Spring Harbor
Press, CSH, NY. RT-PCR is used on an appropriate mRNA sample
selected for the presence of message to produce a cDNA, e.g.,
a monocyte or macrophage cell sample. The original isolate of

10　　IL-1δ was from a whole mouse cDNA library, and for the IL-1ε
from a mouse placenta.

　　　　Full length clones may be isolated by hybridization of
cDNA libraries from appropriate tissues pre-selected by PCR
signal.

15　　As is commonly known, PCR primers are typically designed
to contain at least 15 nucleotides, e.g., 15-30 nucleotides.
The design of IL-1δ or IL-1ε specific primers containing 21
nucleotides, e.g., that code for IL-1δ or ιL-1ε polypeptides
containing at least 4 amino acids from the IL-1δ or IL-1ε

20　　domains are described as follows. Other PCR primers designed
to amplify other IL-1δ or IL-1ε polypeptide fragments will be
designed in a similar fashion, e.g., mutagenesis primers.
Preferably, most or all of the nucleotides in such a primer
encode conserved amino acids, e.g., amino acid residues of SEQ

25　　ID NO: 2 or 4, including IL-1δ or IL-1ε-specific amino acids as
described herein. For example, primers containing at least
40% IL-1δ or IL-1ε-conserved amino acids can be used. Such a
primer, containing 21 nucleotides, can include sequences
encoding at least 3/7, 4/7, 5/7, 6/7 or 7/7 IL-1δ or IL-1ε-

30　　conserved amino acids. Once IL-1δ or IL-1ε amino acids are
selected as templates against which primer sequences are to be
designed, the primers can be synthesized using, e.g., standard
chemical methods. Due to the degeneracy of the genetic code
and the bias of preferred species variants, such primers

should be designed to include appropriate degenerate
sequences, as can be readily determined using common
knowledge.

5   As is described above, IL-1ε or IL-1δ primers, e.g.,
primers based on IL-1ε or IL-1δ specific peptides shown above,
or portions thereof, can be used in PCR reactions to generate
IL-1ε or IL-1δ, probes which can be used in standard screening
methods to identify nucleic acids encoding IL-1ε or IL-1δ
family members (see, e.g., Ausubel, et al., supra).

10

III.   Tissue distribution of IL-1δ or IL-1ε

     Message for the gene encoding IL-1δ has been detected in
a mouse cDNA library. Message for IL-1ε has been detected in
placenta tissue.

15   Southern Analysis: DNA (5 µg) from a primary amplified
cDNA library is digested with appropriate restriction enzymes
to release the inserts, run on a 1% agarose gel and
transferred to a nylon membrane (Schleicher and Schuell,
Keene, NH).

20   Samples for human mRNA isolation could include:
peripheral blood mononuclear cells (monocytes, T cells, NK
cells, granulocytes, B cells), resting (T100); peripheral
blood mononuclear cells, activated with anti-CD3 for 2, 6, 12
h pooled (T101); T cell, TH0 clone Mot 72, resting (T102); T
25   cell, TH0 clone Mot 72, activated with anti-CD28 and anti-CD3
for 3, 6, 12 h pooled (T103); T cell, TH0 clone Mot 72,
anergic treated with specific peptide for 2, 7, 12 h pooled
(T104); T cell, TH1 clone HY06, resting (T107); T cell, TH1
clone HY06, activated with anti-CD28 and anti-CD3 for 3, 6, 12
30   h pooled (T108); T cell, TH1 clone HY06, anergic treated with
specific peptide for 2, 6, 12 h pooled (T109); T cell, TH2
clone HY935, resting (T110); T cell, TH2 clone HY935,
activated with anti-CD28 and anti-CD3 for 2, 7, 12 h pooled
(T111); T cells CD4+CD45RO- T cells polarized 27 days in anti-

CD28, IL-4, and anti IFN-γ, TH2 polarized, activated with anti-CD3 and anti-CD28 4 h (T116); T cell tumor lines Jurkat and Hut78, resting (T117); T cell clones, pooled AD130.2, Tc783.12, Tc783.13, Tc783.58, Tc782.69, resting (T118); T cell random γδ T cell clones, resting (T119); Splenocytes, resting (B100); Splenocytes, activated with anti-CD40 and IL-4 (B101); B cell EBV lines pooled WT49, RSB, JY, CVIR, 721.221, RM3, HSY, resting (B102); B cell line JY, activated with PMA and ionomycin for 1, 6 h pooled (B103); NK 20 clones pooled, resting (K100); NK 20 clones pooled, activated with PMA and ionomycin for 6 h (K101); NKL clone, derived from peripheral blood of LGL leukemia patient, IL-2 treated (K106); NK cytotoxic clone 640-A30-1, resting (K107); hematopoietic precursor line TF1, activated with PMA and ionomycin for 1, 6 h pooled (C100); U937 premonocytic line, resting (M100); U937 premonocytic line, activated with PMA and ionomycin for 1, 6 h pooled (M101); elutriated monocytes, activated with LPS, IFNγ, anti-IL-10 for 1, 2, 6, 12, 24 h pooled (M102); elutriated monocytes, activated with LPS, IFNγ, IL-10 for 1, 2, 6, 12, 24 h pooled (M103); elutriated monocytes, activated with LPS, IFNγ, anti-IL-10 for 4, 16 h pooled (M106); elutriated monocytes, activated with LPS, IFNγ, IL-10 for 4, 16 h pooled (M107); elutriated monocytes, activated LPS for 1 h (M108); elutriated monocytes, activated LPS for 6 h (M109); DC 70% CD1a+, from CD34+ GM-CSF, TNFα 12 days, resting (D101); DC 70% CD1a+, from CD34+ GM-CSF, TNFα 12 days, activated with PMA and ionomycin for 1 hr (D102); DC 70% CD1a+, from CD34+ GM-CSF, TNFα 12 days, activated with PMA and ionomycin for 6 hr (D103); DC 95% CD1a+, from CD34+ GM-CSF, TNFα 12 days FACS sorted, activated with PMA and ionomycin for 1, 6 h pooled (D104); DC 95% CD14+, ex CD34+ GM-CSF, TNFα 12 days FACS sorted, activated with PMA and ionomycin 1, 6 hr pooled (D105); DC CD1a+ CD86+, from CD34+ GM-CSF, TNFα 12 days FACS

sorted, activated with PMA and ionomycin for 1, 6 h pooled
(D106); DC from monocytes GM-CSF, IL-4 5 days, resting (D107);
DC from monocytes GM-CSF, IL-4 5 days, resting (D108); DC from
monocytes GM-CSF, IL-4 5 days, activated LPS 4, 16 h pooled

5   (D109); DC from monocytes GM-CSF, IL-4 5 days, activated TNFα,
monocyte supe for 4, 16 h pooled (D110); leiomyoma L11 benign
tumor (X101); normal myometrium M5 (O115); malignant
leiomyosarcoma GS1 (X103); lung fibroblast sarcoma line MRC5,
activated with PMA and ionomycin for 1, 6 h pooled (C101);

10 kidney epithelial carcinoma cell line CHA, activated with PMA
and ionomycin for 1, 6 h pooled (C102); kidney fetal 28 wk
male (O100); lung fetal 28 wk male (O101); liver fetal 28 wk
male (O102); heart fetal 28 wk male (O103); brain fetal 28 wk
male (O104); gallbladder fetal 28 wk male (O106); small

15 intestine fetal 28 wk male (O107); adipose tissue fetal 28 wk
male (O108); ovary fetal 25 wk female (O109); uterus fetal 25
wk female (O110); testes fetal 28 wk male (O111); spleen fetal
28 wk male (O112); adult placenta 28 wk (O113); and tonsil
inflamed, from 12 year old (X100).

20   Using the information described herein for cloning
species variants, expression of human IL-1ε or IL-1δ can be
determined as above using a human homologue as for a
detectable probe.

        Tissue distribution of transcripts derived from IL-1δ and

25 IL-1ε were determined in experiments using an RNAse protection
assay. Total RNA was prepared from adult brain, spleen, lung,
liver and kidney by homogenization in guanidium thiocyantae
and extraction with phenol, followed by centrifugation through
5.7 M cesium chloride (Sambrook, et al. (1987 and periodic

30 updates) Molecular Cloning: A laboratory Manual Cold Spring
Harbor Laboratory Press, Cold Spring Harbor, New York). Ten
micrograms of total RNA from each tissue, or ten micrograms of
yeast tRNA, was used for RNAse protection assay. Riboprobes
were synthesized using either T7 or T3 RNA polymerase on

linerized templates that were cloned into pBluescript. Each
mouse IL-1δ and IL-1ε probe contained 150-200 nucleotides from
the antisense strand, linked to 25-50 nucleotides of vector
sequence. Reagents were obtained from Ambion (Austin, Texas)
5   following standard manufacturer's protocols.

Tissue distribution of transcripts derived from IL-1δ and
IL-1ε were compared with IL-1γ. The results demonstrate that
IL-1ε expression is detectable in both embryonic, postnatal,
and adult mice. An IL-1ε transcript (about 1.35 kD) is
10   detectable at gestational day 7 and on postnatal day 1, adult
IL-1δ transcripts (about 1.35 kD) were detectable in both the
lung and kidney while not detected in brain, spleen and liver.
For IL-1δ, an approximate 1.35 kD transcript was strongly
detectable at gestational day 15 with a larger sized
15   transcript (approximately 3.5 kD) less strongly detected.
Similar results were observed at postnatal day 1. In adult
tissue, a single sized IL-1δ transcript (approximately 1.8 kD)
was detected in lung, liver, and kidney.

Northern blot: Mouse Northern blots containing
20   approximately 2 μg poly(A)+ RNA per lane, derived from either
total embryo at different days post gestation (Clontech) or
from different tissues (Origene Technologies, Inc.), were
hybridized to the mouse IL-1δ and IL-1ε cDNA probes containing
the complete open reading frames. Probes were labeled with
25   ^{32}P using the Redivue labeling kit (Amersham).
Prehybridization, hybridization, stringency washes and
stripping were carried out according to the manufacturer's
protocols. Membranes were exposed to a phosphorImager.

Quantitation of mRNA expression: A panel of various
30   human skin-derived cell lines or cells, such as fibroblast and
keratinocyte cell lines, and freshly isolated T cells and
Langerhans cells, were used for Taqman-PCR analyses.
Fibroblast and keratinocyte cells were left untreated or were

treated with IL-10 (at 300 ng/ml final), IL-4 (50 ng/ml), or
IFNγ (20 ng/ml) for either 6 or 18 h before RNA was isolated.
RNA was isolated by the guanidium/phenol method. Chomczynski
and Sacchi (1987) <u>Anal. Biochem.</u> 162:156-159. T cell and

5 Langerhans RNA samples were kindly donated by Dr. T.S. Kupper
(Harvard Skin Disease Research Center, Boston, MA). The
reverse transcriptase reactions were performed with
SuperScript^{TM}II (Gibco BRL) according to the supplier's
instructions, except that random hexamers (Promega Corp.), at

10 a final concentration of 1 mM, were added to the reaction.
cDNAs (50 ng per reaction) were analyzed for the expression of
IL-1δ and IL-1ε genes by the Fluorogenic 5'-nuclease PCR assay
(Holland, et al. (1991) <u>Proc. Nat'l Acad. Sci. USA</u> 88:7276-
7280), using a Perkin-Elmer ABI Prism 7700 Sequence Detection

15 System. Reactions were incubated for 2 min at 50° , denatured
for 10 min at 95° C and subjected to 40 two-step amplification
cycles with annealing/extension at 60° C for 1 min followed by
denaturation at 95° C for 15 sec. The amplicons for human IL-
1δ and human IL-1ε were analyzed with FAM-labeled probes. 18S

20 RNA quantities were measured with a VIC-labeled probe and
served as internal controls to normalize for the total amount
of cDNA. Values are expressed as fg/50 ng total cDNA.

IV. Cloning of species counterparts of IL-1δ and IL-1ε

25   Various strategies are used to obtain species
counterparts of mouse IL-1δ and IL-1ε. One method is by cross
hybridization using closely related species DNA probes. The
degree of identity between mouse and human IL-1 counterparts
typically is as high as 70%. It may be useful to go into

30 evolutionarily similar species as intermediate steps. Another
method is by using specific PCR primers based on the
identification of blocks of similarity between human and mouse
IL-1 counterparts, e.g., areas of highly conserved polypeptide
sequence.

     In addition, the IL-1α, IL-1β, and IL-1RA genes cluster
on the same human chromosome. The fourth known member of the
IL-1 family, IL-1γ, which is most closely related to IL-1β, has
been mapped to a different human chromosome. Duplication of
5   the intact IL-1α, IL-1β, IL-1RA gene cluster, a potential
genetic event explaining a proliferation of additional family
members, would suggest the existence of two as yet
unidentified IL-1 genes at the location of the IL-1γ locus.
IL-1δ and IL-1ε are potential candidates, and sequencing of the
10 human IL-1γ locus may well lead to identification of the novel
IL-1 genes.

V.   Production of mammalian IL-1δ protein

     An appropriate, e.g., GST, fusion construct is engineered
15 for expression, e.g., in E. coli. For example, a mouse IGIF
pGex plasmid is constructed and transformed into E. coli.
Freshly transformed cells are grown in LB medium containing 50
μg/ml ampicillin and induced with IPTG (Sigma, St. Louis, MO).
After overnight induction, the bacteria are harvested and the
20 pellets containing IL-1δ are isolated. The pellets are
homogenized in TE buffer (50 mM Tris-base pH 8.0, 10 mM EDTA
and 2 mM pefabloc) in 2 liters. This material is passed
through a microfluidizer (Microfluidics, Newton, MA) three
times. The fluidized supernatant is spun down on a Sorvall
25 GS-3 rotor for 1 h at 13,000 rpm. The resulting supernatant
containing the IL-1δ is filtered and passed over a
glutathione-SEPHAROSE column equilibrated in 50 mM Tris-base
pH 8.0. The fractions containing the IL-1δ-GST fusion protein
are pooled and cleaved with thrombin (Enzyme Research
30 Laboratories, Inc., South Bend, IN). The cleaved pool is then
passed over a Q-SEPHAROSE column equilibrated in 50 mM Tris-
base. Fractions containing IL-1δ are pooled and diluted in
cold distilled H_2O, to lower the conductivity, and passed back

over a fresh Q-SEPHAROSE column. Fractions containing IL-1δ
are pooled, aliquoted, and stored in the -70° C freezer.

   Comparison of the CD spectrum with mouse IL-1β may
suggest that the protein is correctly folded. See Hazuda, et
5   al. (1969) J. Biol. Chem. 264:1689-1693.

   Similar techniques will be applicable to a full length
IL-1ε.

   Protein expression and purification of human IL-1δ and
IL-1ε: Adenoviral vectors containing full-length human IL-1δ
10   and IL-1ε were constructed by PCR and used to transfect Q293
packaging cells. Produced viruses were subsequently purified,
with all procedures according to manufacturer's protocols
(Invitrogen). IL-1 proteins were prepared from 5×10^8
infected Q293 cells (adenoviruses at 10 MOI) which were
15   subsequently incubated for 5 days in a cell factory in a total
volume of 1 1 of serum-free CMF-1 medium (Gibco BRL). Culture
medium was dialyzed (Spectra/Por membrane tubing, MW cut off:
6-8 kD) against 50 mM Tris-HCl, pH 8.0, 1 mM EDTA, and
subsequently passed over Hitrap Q sepharose and Heparin
20   columns. The flow-through, containing the IL-1 proteins, was
sterile-filtered and concentrated approximately 70 times using
an Amicon 8400 ultrafiltration cell with a 10 kD MW cut off
membrane. The samples were dialyzed against PBS, and the
protein content quantified by PAGE and Coomassie Blue staining
25   using lysozyme as a standard. Protein identities were
confirmed by N-terminal sequencing. Identically treated Q293
cells infected with adenovirus encoding green fluorescent
protein provided a mock control. Endotoxin levels were
determined using the Limulus Amebocyte Lysate assay
30   (BioWhittaker) and were less then 4 EU/ml. Protein samples
were stored at 4° C.

   Expression vectors: For mammmalian expression, vectors
encoding full-length human R1, mouse R3, mouse R4 and human R5
were constructed by inserting PCR-generated cDNA fragments

into pME18S. Kitamura, et al. (1991) <u>Proc. Nat'l Acad. Sci.</u>
<u>USA</u> 88:5082-5086. Human IL-1R6 was a generous gift of Dr.
R.A. Maki (Neurocrine Biosciences, Inc., San Diego, CA), and
both human IL-1R6 and IL-1R7 cDNA were subcloned directly into
5   pME18S. For secretion studies, full-length mouse IL-1δ and
IL-1ε were fused to a C-terminal E-tag and put into pCDM8
(Stratagene Cloning Systems) via PCR. pCDM8 constructs
containing mouse IL-1δ or IL-1ε fused to an N-terminal
exogenous SLAM leader (Castro, et al. (1999) <u>J. Immunol.</u>
10   163:5860-5870), and the extracellular part of mouse IL-1R7
fused to a C-terminal IgG module served a positive controls.
The reporter gene plasmid pNFκB-Luc (Stratagene) contains five
NFκB sites and a basic promoter element to drive luciferase
expression, and pRSV-βGal results in constitutive expression
15   of β-galactosidase.

VI.   Secretion studies
       293-T cells were transfected with mouse IL-1δ and IL-1ε
pCDM8:E-tag expression constructs with and without an N-
20   terminal exogenous SLAM leader sequence using Ca$_3$(PO$_4$)$_2$ (5
Prime) according to the supplier's protocol. Cells were
cultured in CMF-1 medium. Seventy-two hours post
transfection, supernatants and lysates were collected, and
screened for E-tagged proteins by Western blotting according
25   to standard procedures.

VII. Signal pathway analysis
       Reporter assay: For reporter gene assays, Jurkat E6.1
cells (4 x 10^6) were transiently transfected by
30   electroporation (300 V, 960 microfarad) with 2 μg pNFκB-Luc
reporter gene plasmid, 0.5 μg of pRSV-βGal and 4 μg of each
receptor-encoding DNA. Twenty hours post-transfection, cells
were stimulated with 20 ng/ml of human IL-1α, IL-1β, IL-18/IL-

1γ, or IL-1RA, or 50 ng/ml human IL-1δ or IL-1ε for 6 h. Cells
were lysed using Reporter Lysis Buffer (Promega) and
luciferase and β-galactosidase activities were assessed using
Luciferase Assay Reagent (Promega) and Galacto-Light Kit

5   (Tropix), respectively. Luciferase activities (in RLU) were
normalized on the basis of β-galactosidase activities. For
inhibition studies of IL-1R1-mediated activation of NFκB, IL-
1α was used at 50 pg/ml and IL-1RA and IL-1δ were used at
concentrations ranging from 10 μg/ml to 10 pg/ml. Inhibition

10 of the IL-1R6-mediated response was done with IL-1ε used at 50
ng/ml and IL-1δ and IL-1RA at concentrations ranging from 10
μg/ml to 64 pg/ml.

        The ability of the novel IL-1s to initiate IL-1R-
mediated signaling was studied via an NFκB-dependent reporter

15 assay using ligand-stimulated Jurkat T cells transiently
transfected with different pairs of IL-1Rs. The R1/R3
combination, conferring responsiveness to IL-1α and IL-1β
ʾ(Cullinan et al. (1998) J. Immunol. 161:d5614-5620; and
Greenfeder et al. (1995a) J. Biol. Chem. 270:13757-13765), did

20 not generate a response to IL-1δ or IL-1ε. Also, the R5/R7
combination, required to mediate a response to IL-18 (Born et
al. (1998) J. Biol. Chem. 273:29445-29450), did not result in
signaling upon addition of the novel IL-1 ligands. The next
step was to test the orphan IL-1R-like molecules IL-1R4 and

25 IL-1R6, classified as potential ligand-binding receptors based
on their homology to IL-1R1 (Sana, et al. (2000) Genomics
69:252-262), in combination with various other IL-1R-like
molecules, i.e., IL-1R3, IL-1R7, IL-1R9 and IL-1R10
(classified as potential signaling receptors based on their

30 homology to IL-1R3 (Sana, et al. supra.) for their potential
to confer responsiveness to IL-1δ and IL-1ε. Data consistently
showed an IL-1R6-mediated activation of NFκB upon stimulation
with IL-1ε, but not IL-1δ or the mock control.

In line with the striking similarity between IL-1δ and
IL-1ra, the possibility of IL-1δ being an antagonist of IL-1
responses rather than being an IL-1 agonist was tested. Using
the same reporter assay, it was shown that IL-1ra but not IL-
1δ is able to antagonize the IL-1R1-mediated activation of
NFκB upon stimulation with IL-1α. Vice versa, IL-1δ but not
IL-1ra, is able to antagonize the IL-1R6-mediated activation
of NFκB upon stimulation with IL-1e. Importantly, IL-1ra shows
a 50% inhibition of the IL-1R1-mediated response to IL-1α at
about a 1000-fold excess over IL-1α, whereas IL-1δ results in
a similar inhibition of the IL-1R6 response at concentrations
similar to or even less than IL-1ε.

In lesional psoriasis skin, characterized by chronic
cutaneous inflammation, the expression of the IL-1 ligands IL-
1δ and IL-1ε and their corresponding receptor IL-1R6 are all
significantly increased relative to skin from a healthy
individual. The increase is most prominent for IL-1ε, in line
with in vitro-cultured keratinocytes stimulated with the pro-
inflammatory cytokines IL-1β/TNFα. Activation of PBMC also
leads to increased levels of both IL-1 ligands and their
receptor, albeit to a lesser extent than observed in lesional
psoriasis skin.

VIII. Biological Assays with IL-1δ or IL-1ε

Biological assays confirmed IFN-γ inducing activity by
IL-1γ on T cells. IL-1γ stimulates production of IFN-γ by
purified NK cells, and that induction is strongly synergized
with IL-12 or IL-2. Similar biological activity should be
exhibited by IL-1δ and/or IL-1ε or their antagonists.

The family of interleukins 1 contains molecules, each of
which is an important mediator of inflammatory disease. For a
comprehensive review, see Dinarello (1996) "Biologic basis for
interleukin-1 in disease" Blood 87:2095-2147. There are
indications that the various IL-1's play important roles in

the initiation of disease, including the recently identified
IGIF/IL-1γ (e.g., Rothe, et al. (1997) "Active stage of
autoimmune diabetes is associated with the expression of a
novel cytokine, IGIF, which is located near Idd2." J. Clin.
5   Invest. 99:469-474. The finding of novel proteins related to
the IL-1 family furthers the identification of molecules that
provide the molecular basis for initiation of disease and
allow for the development of therapeutic strategies of
increased range and efficacy.

10   Similar biological assays as applied to other known
members of the family should be performed with purified IL-1δ
or IL-1ε.

IX. Preparation of antibodies specific for IL-1δ or IL-1ε

15   Inbred Balb/c mice are immunized intraperitoneally with
recombinant forms of the protein, e.g., purified soluble IL-
1δ— or IL-1ε-FLAG or stable transfected NIH-3T3 cells. Animals
are boosted at appropriate time points with protein, with or
without additional adjuvant, to further stimulate antibody
20   production. Serum is collected, or hybridomas produced with
harvested spleens.

         Alternatively, Balb/c mice are immunized with cells
transformed with the gene or fragments thereof, either
endogenous or exogenous cells, or with isolated membranes
25   enriched for expression of the antigen. Serum is collected at
the appropriate time, typically after numerous further
administrations. Various gene therapy techniques may be
useful, e.g., in producing protein in situ, for generating an
immune response.

30   Monoclonal antibodies may be made. For example,
splenocytes are fused with an appropriate fusion partner and
hybridomas are selected in growth medium by standard
procedures. Hybridoma supernatants are screened for the
presence of antibodies which bind to the desired IL-1γ, e.g.,

by ELISA or other assay. Antibodies which specifically recognize IL-1δ or IL-1ε may also be selected or prepared.

In another method, synthetic peptides or purified protein are presented to an immune system to generate monoclonal or polyclonal antibodies. See, e.g., Coligan (1991) Current Protocols in Immunology Wiley/Greene; and Harlow and Lane (1989) Antibodies: A Laboratory Manual Cold Spring Harbor Press. In appropriate situations, the binding reagent is either labeled as described above, e.g., fluorescence or otherwise, or immobilized to a substrate for panning methods. Nucleic acids may also be introduced into cells in an animal to produce the antigen, which serves to elicit an immune response. See, e.g., Wang, et al. (1993) Proc. Nat'l. Acad. Sci. 90:4156-4160; Barry, et al. (1994) BioTechniques 16:616-619; and Xiang, et al. (1995) Immunity 2: 129-135.

X.   Production of fusion proteins with IL-1δ or IL-1ε

Various fusion constructs are made with IL-1δ or IL-1ε. This portion of the gene is fused to an epitope tag, e.g., a FLAG tag, or to a two hybrid system construct. See, e.g., Fields and Song (1989) Nature 340:245-246.

The epitope tag may be used in an expression cloning procedure with detection with anti-FLAG antibodies to detect a binding partner, e.g., receptor for the respective IL-1. The two hybrid system may also be used to isolate proteins which specifically bind to IL-1δ or IL-1ε.

XI. Isolation of a Receptor for IL-1δ or IL-1ε

An IL-1δ can be used as a specific binding reagent to identify its binding partner, by taking advantage of its specificity of binding, much like an antibody would be used. A binding reagent is either labeled as described above, e.g., fluorescence or otherwise, or immobilized to a substrate for panning methods.

The binding composition is used to screen an expression
library made from a cell line which expresses a binding
partner, i.e., receptor. Standard staining techniques are
used to detect or sort intracellular or surface expressed
5   receptor, or surface expressing transformed cells are screened
by panning. Screening of intracellular expression is
performed by various staining or immunofluorescence
procedures. See also McMahan, et al. (1991) EMBO J. 10:2821-
2832.

10   For example, on day 0, precoat 2-chamber permanox slides
with 1 ml per chamber of fibronectin, 10 ng/ml in PBS, for 30
min at room temperature. Rinse once with PBS. Then plate COS
cells at 2-3 x 105 cells per chamber in 1.5 ml of growth
media. Incubate overnight at 37° C.

15   On day 1 for each sample, prepare 0.5 ml of a solution of
66 µg/ml DEAE-dextran, 66 µM chloroquine, and 4 µg DNA in
serum free DME. For each set, a positive control is prepared,
e.g., of IL-1γ-FLAG cDNA at 1 and 1/200 dilution, and a
negative mock. Rinse cells with serum free DME. Add the DNA
20   solution and incubate 5 hr at 37° C. Remove the medium and
add 0.5 ml 10% DMSO in DME for 2.5 min. Remove and wash once
with DME. Add 1.5 ml growth medium and incubate overnight.

On day 2, change the medium. On days 3 or 4, the cells
are fixed and stained. Rinse the cells twice with Hank's
25   Buffered Saline Solution (HBSS) and fix in 4% paraformaldehyde
(PFA)/glucose for 5 min. Wash 3X with HBSS. The slides may
be stored at -80° C after all liquid is removed. For each
chamber, 0.5 ml incubations are performed as follows. Add
HBSS/saponin (0.1%) with 32 µl/ml of 1 M NaN3 for 20 min.
30   Cells are then washed with HBSS/saponin 1X. Add appropriate
IL-1δ or IL-1δ/antibody complex to cells and incubate for 30
min. Wash cells twice with HBSS/saponin. If appropriate, add
first antibody for 30 min. Add second antibody, e.g., Vector
anti-mouse antibody, at 1/200 dilution, and incubate for 30

min.   Prepare ELISA solution, e.g., Vector Elite ABC
horseradish peroxidase solution, and preincubate for 30 min.
Use, e.g., 1 drop of solution A (avidin) and 1 drop solution B
(biotin) per 2.5 ml HBSS/saponin. Wash cells twice with

5   HBSS/saponin. Add ABC HRP solution and incubate for 30 min.
Wash cells twice with HBSS, second wash for 2 min, which
closes cells. Then add Vector diaminobenzoic acid (DAB) for 5
to 10 min. Use 2 drops of buffer plus 4 drops DAB plus 2
drops of H_2O_2 per 5 ml of glass distilled water. Carefully

10   remove chamber and rinse slide in water. Air dry for a few
minutes, then add 1 drop of Crystal Mount and a cover slip.
Bake for 5 min at 85-90° C.

        Evaluate positive staining of pools and progressively
subclone to isolation of single genes responsible for the

15   binding.

        Alternatively, IL-1δ reagents are used to affinity purify
or sort out cells expressing a receptor. See, e.g., Sambrook,
et al., or Ausubel, et al.

        Another strategy is to screen for a membrane bound

20   receptor by panning. The receptor cDNA is constructed as
described above. The ligand can be immobilized and used to
immobilize expressing cells. Immobilization may be achieved
by use of appropriate antibodies which recognize, e.g., a FLAG
sequence of a IL-1δ fusion construct, or by use of antibodies

25   raised against the first antibodies. Recursive cycles of
selection and amplification lead to enrichment of appropriate
clones and eventual isolation of receptor expressing clones.

        Phage expression libraries can be screened by mammalian
IL-1δ. Appropriate label techniques, e.g., anti-FLAG

30   antibodies, will allow specific labeling of appropriate
clones.

        All citations herein are incorporated herein by reference to
the same extent as if each individual publication or patent

application was specifically and individually indicated to be incorporated by reference including all figures and drawings.

    Many modifications and variations of this invention, as will be apparent to one of ordinary skill in the art can be

5 made to adapt to a particular situation, material, composition of matter, process, process step or steps, to preserve the objective, spirit and scope of the invention. All such modifications are intended to be within the scope of the claims appended hereto without departing from the spirit and

10 scope of the invention. The specific embodiments described herein are offered by way of example only, and the invention is to be limited by the terms of the appended claims, along with the full scope of equivalents to which such claims are entitled; and the invention is not to be limited by the

15 specific embodiments that have been presented herein by way of example.